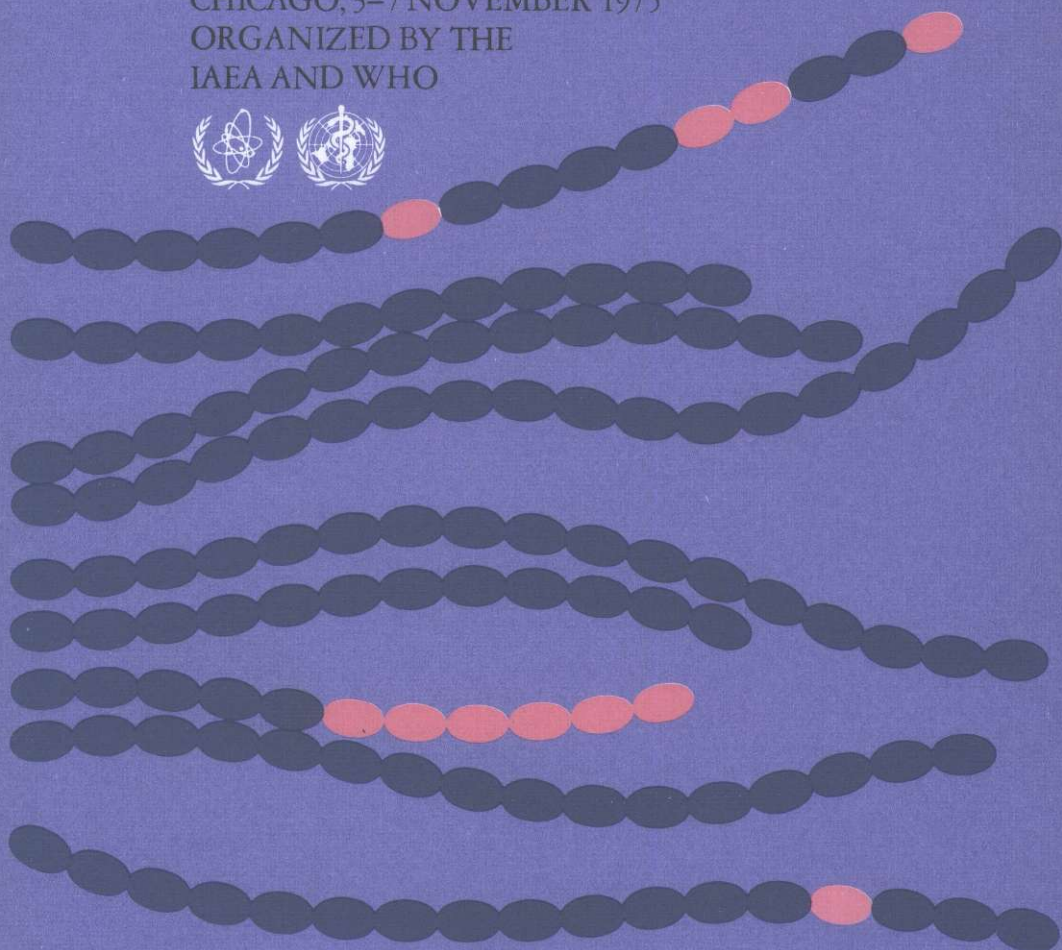


Biological and Environmental Effects of Low-Level Radiation

Vol. II

PROCEEDINGS OF A
SYMPOSIUM
CHICAGO, 3-7 NOVEMBER 1975
ORGANIZED BY THE
IAEA AND WHO



INTERNATIONAL ATOMIC ENERGY AGENCY, VIENNA, 1976

The cover illustration is an example of radiation-induced somatic mutation: change in colour from purplish-blue to purplish-red in stamen hairs of a flower, Clone 02 *Tradescantia*, which has a highly radiosensitive biological system. Designed from an original microscopic photograph by R.P. and L.W. Mericle (see Vol.I, p.31).

BIOLOGICAL
AND ENVIRONMENTAL EFFECTS
OF LOW-LEVEL RADIATION

VOL.II

PROCEEDINGS SERIES

BIOLOGICAL
AND ENVIRONMENTAL EFFECTS
OF LOW-LEVEL RADIATION

PROCEEDINGS OF A SYMPOSIUM
ON BIOLOGICAL EFFECTS OF LOW-LEVEL RADIATION
PERTINENT TO PROTECTION OF MAN AND HIS ENVIRONMENT
ORGANIZED BY
THE INTERNATIONAL ATOMIC ENERGY AGENCY
AND THE WORLD HEALTH ORGANIZATION
AND HELD IN
CHICAGO, 3 - 7 NOVEMBER 1975

In two volumes

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FOREWORD

Public concern is being expressed over the effects on man and his environment of exposure to low levels of ionizing radiation from the increasing use of atomic energy in medicine and industry, and particularly from the projected expansion of nuclear power generation. This concern arises in spite of the fact that national and international measures taken to prevent radioactive pollution from nuclear industries are far more advanced than those against chemical pollutants from conventional industries, and although only a very small fraction of the overall radiation dose to man and environment comes from the nuclear industry.

The biological effects of radiation have been fairly well defined for exposures at high doses. However, there are still considerable uncertainties regarding both genetic and somatic effects at low radiation doses and low dose rate that might be relevant to occupational and public health. As pointed out by Hellmut Glubrecht, Deputy Director General, Department of Research and Isotopes, IAEA, in his opening address, the emphasis of the work yet to be done in radiation biology has to be shifted to the low dose range and especially to the effects of small amounts of incorporated radioactive materials. The methods of such research must be different from the conventional statistical evaluation and should aim at a better understanding of the basic molecular processes underlying the radiation effects. It is not to be expected that low dose effects are qualitatively of the same nature as effects of higher radiation doses.

A symposium was therefore convened in Chicago by the International Atomic Energy Agency and the World Health Organization with the co-operation of the United States Energy Research and Development Administration and Argonne National Laboratory from 3 to 7 November 1975. The Symposium on the Biological Effects of Low-Level Radiation Pertinent to Protection of Man and his Environment was attended by 281 participants and observers from twenty-one countries and five international organizations. It was planned to review the present understanding of biological effects of low-level ionizing radiation from external and internal sources at cellular, whole-organism and population levels. The principal consideration was how to obtain valid numerical estimates of the risk to man and his environment of low doses of ionizing radiation in the range of, say, a few rads or fractions of a rad annually, i.e. doses of the order of natural background radiation or small multiples thereof.

Seventy-two papers were presented in nine sessions, covering genetic and somatic effects of low-level radiation, effects of internal exposure, especially by transuranic elements, epidemiological studies, theoretical or mathematical models, and — most important of all — risk estimation in human populations. In addition, two invited evening lectures were given. One, by M. Eisenbud of New York University, on areas of high natural background radiation, was a comprehensive rapporteur's report of the International Symposium on Areas of High Natural Radioactivity held in Brazil in June 1975, the proceedings of which will be published by the Brazilian Academy of Science (the report is therefore not included in these Proceedings). The second evening lecture presented the principal results which the former ABCC (Atomic Bomb Casualty Commission) in Japan had developed on the incidence of such malignancies as leukaemia, thyroid tumours, breast and lung tumours, and on the future programme of the Radiation Effects Research Foundation, a new organization which replaces the ABCC. This lecture was in fact a joint presentation by H. Yamashita of Keio University, Japan, Chairman of the Board of Directors of the RERF, and V.P. Bond, of Brookhaven National Laboratory, United States of America. The full text of the papers and discussions, together with the general summaries given at the close of the meeting by three invited experts, is published in these Proceedings, which appear in two volumes. It is hoped that the Proceedings will be used as a major source of reference conveying the status of knowledge in 1975 on this important subject.

The IAEA and WHO gratefully acknowledge the assistance and co-operation of the Government of the United States of America and particularly the US Energy Research and Development Administration and Argonne National Laboratory for acting as hosts and providing the excellent facilities that contributed so much to the successful outcome of the meeting. Special mention is due to the members of the United States Scientific Programme Committee, headed by W.K. Sinclair (Argonne National Laboratory), who offered their generous co-operation during the preparation and running of the Symposium, especially in the selection of USA papers, for which they were responsible. The Committee, under W.K. Sinclair, comprised K.H. Clifton (University of Wisconsin), H.S. Ducoff (University of Illinois), M.M. Elkind (Argonne National Laboratory), R.J.M. Fry (Argonne National Laboratory), M.L. Griem (University of Chicago), R.E. Rowland (Argonne National Laboratory), and M. Schulman (Division of Biomedical and Environmental Research, US Energy Research and Development Administration).

EDITORIAL NOTE

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INTERNAL EXPOSURE
(Sessions V, VI and VII)

Co-Chairmen:

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INFLUENCE OF VARIATIONS IN DOSE AND DOSE RATES ON BIOLOGICAL EFFECTS OF INHALED BETA-EMITTING RADIONUCLIDES

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Abstract

INFLUENCE OF VARIATIONS IN DOSE AND DOSE RATES ON BIOLOGICAL EFFECTS OF INHALED BETA-EMITTING RADIONUCLIDES.

The biological effects of inhaled β -emitting radionuclides, ^{90}Y , ^{91}Y , ^{144}Ce and ^{90}Sr , are being investigated in beagle dogs that received single acute exposures at 12 to 14 months of age. The aerosols studied have included $^{91}\text{YCl}_3$, $^{144}\text{CeCl}_3$, $^{90}\text{SrCl}_2$, and ^{90}Y , ^{91}Y , ^{144}Ce or ^{90}Sr in aluminosilicate particles. Each aerosol, when inhaled and deposited in the lung, resulted in distinctive radiation dose patterns related to the physical half-life of the radionuclide under study and the biological pattern of retention and translocation of the particles or their dissolution products. Thus, $^{91}\text{YCl}_3$, $^{144}\text{CeCl}_3$ and the aluminosilicate containing radionuclide particles all resulted in significant exposures to lung; $^{91}\text{YCl}_3$, $^{144}\text{CeCl}_3$ and $^{90}\text{SrCl}_2$ resulted in significant exposures to bone; $^{91}\text{YCl}_3$ and $^{144}\text{CeCl}_3$ resulted in significant exposures to liver. The biological effects observed may be related directly to the tissues irradiated. The higher initial dose-rate exposures have been more effective than low dose-rate exposures on a per-rad basis in producing early effects. When the initial high dose rate decreases rapidly, early deaths are avoided and the total dose accumulated is less than that accumulated with protracted lower dose rates. To date (<5 years post-inhalation exposure) only a limited number of neoplasms have been observed in these animals. In animals with protracted lower dose-rate exposure to bone, liver and lung, late-occurring neoplasms have frequently been observed at relatively high total doses. The current status of these long-term studies is reported with emphasis placed on discussion of the influence of total dose and dose rate on the incidence and nature of late-occurring cancer of the lung and other tissues. In other studies on the toxic effects of a single inhalation exposure of mice to $^{144}\text{CeO}_2$, it was observed that, on a μCi initial lung burden per kilogram body weight basis, mice did not develop pulmonary tumours whereas beagle dogs did. To determine whether this observation was due to the shorter half-time of the retention of ^{144}Ce in relatively insoluble forms in the lung of mice compared with the beagle dog, or if there was a species variation in the response to β radiation, mice have been repeatedly exposed by inhalation to $^{144}\text{CeO}_2$ to maintain lung burdens of ^{144}Ce that resulted in radiation dose rates similar to that observed in beagle dogs. Several of the repeatedly exposed mice developed malignant pulmonary tumours. Thus, with similar dose rates and cumulative doses to the lung, mice and dogs responded in a similar manner to chronic β radiation.

INTRODUCTION

The potential for human exposures to beta-emitting radionuclides that may be accidentally released from various stages of the nuclear fuel cycle dictates concern for the relationship between radiation dose and the resultant biomedical response. Such dose-response data are needed as input to (a) developing

standards that provide for maximum safety and effectiveness in the conduct of nuclear operations, and (b) estimating the health consequence costs of nuclear operations. In the absence of adequate dose-response data on inhaled beta-emitting radionuclides in man, it is necessary to obtain such information in experimental animal studies. In designing experimental animal studies, a choice must be made between attempting to simulate various accidental releases or obtaining more basic data that will establish the importance of various factors that may influence dose-response relationships. In this Institute, emphasis has been placed on the latter approach. Specifically, studies are being conducted with seven different aerosols ($^{91}\text{YCl}_3$, $^{144}\text{CeCl}_3$, $^{90}\text{SrCl}_2$, and ^{90}Y , ^{91}Y , ^{144}Ce or $^{90}\text{Sr}^1$ in fused aluminosilicate particles) that produce markedly different radiation dose patterns to the lung, skeleton and liver of the Beagle dog.

In addition, $^{144}\text{CeO}_2$, a relatively insoluble material similar to ^{144}Ce in fused aluminosilicate particles, is being studied in mice. It is cleared more rapidly from the mouse lung than the relatively insoluble ^{144}Ce is cleared from the dog lung; thus the dose pattern received by the lung of the two species is different. In a study of the toxicity of inhaled $^{144}\text{CeO}_2$ in mice reported in 1974 [1], no malignant primary pulmonary neoplasms were observed in mice that had initial lung burdens of ^{144}Ce that were similar to those that produced malignant lung neoplasms in dogs. Speculation was presented as to the possible importance of the species differences in dose pattern as a factor in the apparent difference in dose-response relationship. To hold the variable of dose pattern constant, mice were repeatedly exposed to $^{144}\text{CeO}_2$, thereby achieving similar beta dose patterns to lung for the two species. With dose pattern no longer a variable, it is possible to determine if the dose-response relationships for the two species are similar. The current status of these continuing studies is reviewed.

METHODS

The experimental approach and design of the Beagle dog studies have been described [2,3]. The experimental design is shown in Table I. Equal numbers of male and female Beagle dogs, born and raised in this colony, were given single, brief (< 1 hr) nose-only exposures to radioactive aerosols or were sham exposed at 12-14 months of age. The exposure techniques have been previously described [4-6]. The initial lung burden of radioactivity was controlled for individual dogs by varying the radioactivity of the aerosol or the

¹ ^{144}Ce or ^{90}Sr as used in this text refer to ^{144}Ce in equilibrium with its daughter ^{144}Pr , or ^{90}Sr in equilibrium with its daughter ^{90}Y .

duration of the exposure. The aerosols of $^{90}\text{SrCl}_2$, $^{91}\text{YCl}_3$ or $^{144}\text{CeCl}_3$ were prepared by adding the appropriate radionuclide to a carrier solution of CsCl from which an aerosol was generated using a Lovelace nebulizer. The fused aluminosilicate particles were prepared by cation exchange of the radionuclide into montmorillonite clay, generated from a suspension in distilled water using a Lovelace nebulizer and heat-treated by passing through a heating column operated at 1100°C . The aerosols were characterized by examination of samples collected by an electrostatic precipitator, a cascade impactor, and by radioanalysis of air filter samples. The particle size distributions were poly-disperse and could be described by log-normal functions with activity median aerodynamic diameters ranging from 0.8 to $2.7\text{ }\mu\text{m}$ and with geometric standard deviations of 1.4 to 2.7. Control dogs inhaled similar heat-treated particles containing a stable form of the elements being studied, and deposited an amount of fused clay (100-400 μg in the lung) approximately equal to the amount inhaled and deposited by dogs exposed to the radionuclides in fused clay particles.

The mouse studies were conducted with conventionally reared C57BL/6J female mice obtained from Jackson Laboratories, Bar Harbor, Maine, USA. They were housed 3 per cage and were given food (Wayne Lab-Blox, Allied Mills, Inc., Chicago, Ill.) and water ad libitum. The mice were exposed to aerosols of $^{144}\text{CeO}_2$ prepared by two-stage heat treatment (390°C and 850°C) of droplets generated from a suspension of $^{144}\text{CeCl}_3$ in 0.6 M HCl (4 mg Ce/ml). After passing through the heating column, the aerosol was mixed with dry, cool diluting air and passed through an exposure chamber resulting in essentially nose-only exposure of up to 84 mice [7]. The activity median aerodynamic diameter of the $^{144}\text{CeO}_2$ particles ranged from 1.2 to $1.5\text{ }\mu\text{m}$ with a geometric standard deviation that ranged from 1.4 to 1.7. Exposures ranged up to 25 minutes in duration. The experimental design for the mouse studies is shown in Table II. Lung burden levels for mice were selected to provide cumulative doses that were similar to those that resulted in large numbers of lung neoplasms in Beagle dogs.

Whole-body counting was performed on each animal immediately after exposure and at intervals thereafter to determine the total amount of aerosol deposited in the body during exposure and its subsequent retention. The amount of aerosol initially deposited in the pulmonary region, the initial lung burden (ILB), was also determined from the whole-body counting data. This was accomplished by fitting multicomponent exponential functions to the data and considering the ILB to be the fraction of the initial body burden

Table I
Experimental Design for Dose-Response Studies in Beagle Dogs Given a Single Brief Exposure
at 12-14 Months of Age to a Radioactive Aerosol (Status as of 10/1/75)

Radionuclide and Form	Number of Dogs	Range of Initial Lung Burdens ($\mu\text{Ci/kg}$ Body Weight)		Approximate Effective Half-Life in Whole Body	Approximate Effective Half-Life in Lung	Organs Receiving Significant Radiation Dose		
						Lung	Skeleton	Liver
$^{90}\text{SrCl}_2$	66	1	- 190	5-10 yrs	minutes		+++	
$^{91}\text{YCl}_3$	46	14	- 540	59 days	hours to days	++	++	+
$^{144}\text{CeCl}_3$	55	2.6	- 360	284 days	hours to months	+++	+++	+++
^{90}Y in FAP*	89	80	- 5200	2.6 days	2.6 days	++++		
^{91}Y in FAP	96	11	- 360	53 days	50 days	++++		
^{144}Ce in FAP	111	0.0024	- 210	200 days	180 days	++++	+	+
^{90}Sr in FAP	106	0.2	- 94	400 days	400 days	++++	+	
Controls (composite)	111							

*FAP - in fused aluminosilicate particles.

Table II

Experimental Design for Studies of the Effect of Single or Repeated Exposures of Mice to Aerosols of $^{144}\text{CeO}_2$

Groups of Mice	Desired Exposure Times (Approximate Age in Days)						
	70	130	190	250	310	370	430
I. <u>Nontreated:</u>	270*						
II. <u>Single Exposure:</u>							
A. Sham Exposed	120						
B. Stable CeO_2 Exposed	120						
C. 0.2 μCi ILB**	76						
D. 1.0 μCi ILB	320						
E. 4.5 μCi ILB	409						
III. <u>Repeated Exposures:</u>							
A. Sham Exposed	156	148	148	147	141	140	134
B. Stable CeO_2 Exposed	160	155	154	149	145	144	143
C. 0.2 μCi ILB	164	156	156	153	153	153	150
D. 1.0 μCi ILB	163	157	154	153	146	130	114
E. 4.5 μCi ILB	165	144	61	8	0	0	0

* Number of mice per group.

** Initial lung burdens after single or repeated inhalation exposures.

associated with all but the first, rapidly clearing, component associated with clearance of the upper respiratory tract and tracheobronchial region [8].

The mean organ-absorbed dose rates and cumulative doses were calculated for each animal using its own whole-body retention data and the relationships between organ burden and total burden determined in various parallel studies. The equation for cumulative absorbed beta radiation dose is

$$\text{cumulative rads} = \frac{0.0512 \bar{E}(\text{AF})A_0}{W(\text{TF})} \int_0^t \text{LuB}(t)dt$$

\bar{E} = average β energy in MeV

AF = fractional absorption of β energy = 1.0 for dog lung, 0.35 for mouse lung

A_0 = ILB in μCi

W = body weight at exposure in kg

TF = lung weight with blood as fraction of body weight = 0.011

LuB(t) = lung burden in μCi as function of days after exposure

Each dog was given a physical examination annually. All dogs and mice were observed daily until death occurred spontaneously or the animals were

euthanized in a moribund condition. Complete gross and histopathologic examinations were performed at death.

RESULTS

Dog Studies

The numbers of dogs and ranges of initial lung burdens for the dogs are shown in Table I. Although a series of discrete activity levels was used for planning the inhalation exposures, variability among dogs and exposure conditions produced a spread in initial body and lung burdens at each level. Each entire experiment contains a rather continuous spectrum of ILB values within the range listed. The radionuclides and aerosol forms used resulted in seven different patterns of radiation dose which are summarized in Table I as to the organs receiving significant doses. In addition, the dose pattern to each organ varied related to differences in the effective retention of the different radionuclides and aerosol forms in the organ. This is illustrated in Figures 1 and 2 for lung.

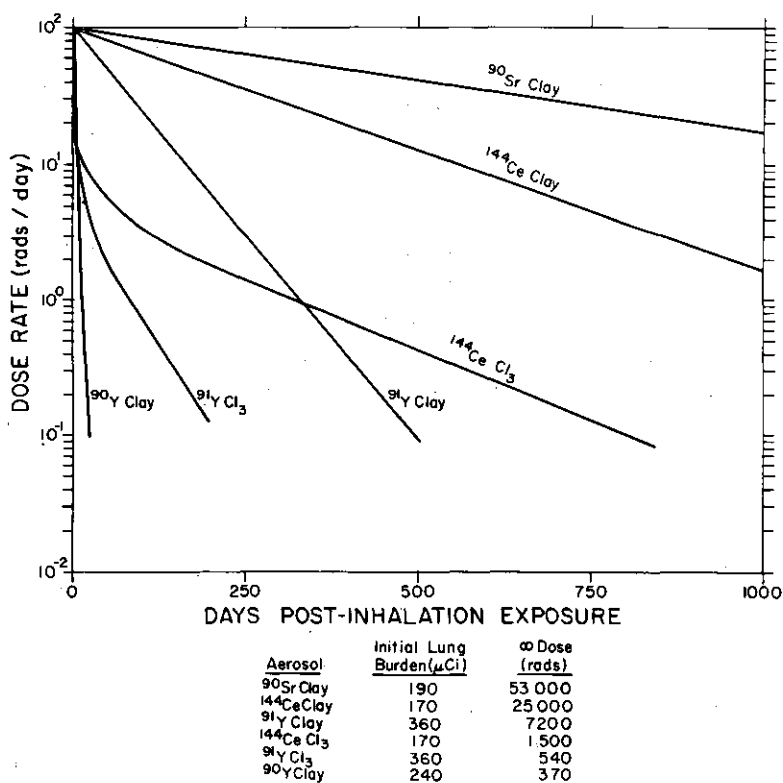


FIG. 1. Absorbed β dose rate to lung for beagles for various inhaled radioactive aerosols normalized to 100 rad/d initial dose rate (110-g lung).

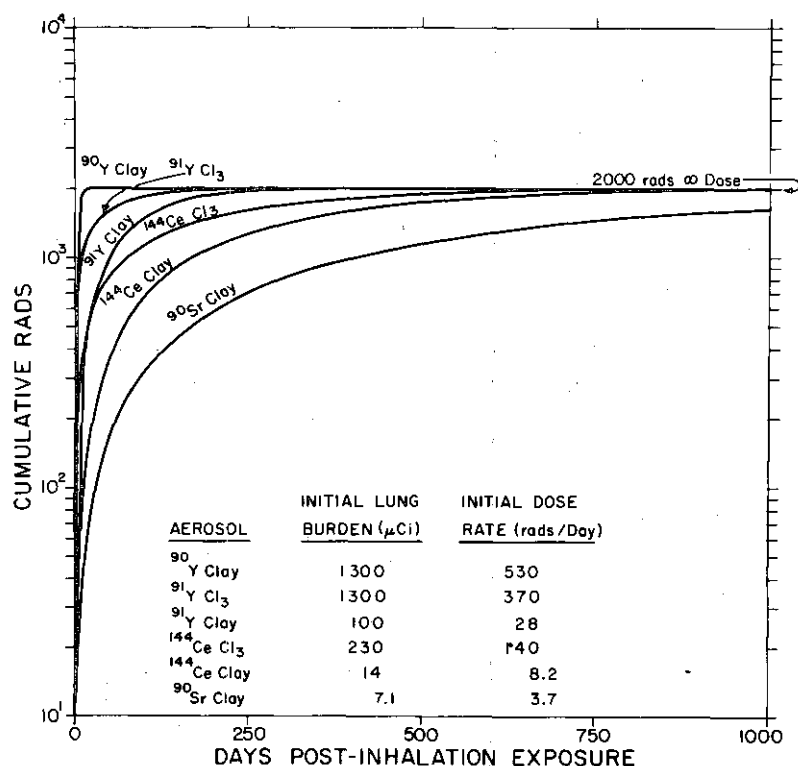


FIG. 2. Patterns for accumulating 2000 rads total β dose to lung in beagles from various inhaled radioactive aerosols (110-g lung).

The spectrum of disease observed within a specific study is illustrated in Figure 3, where the survival of dogs that inhaled $^{144}\text{CeCl}_3$ is related to their long-term retained burden (initial lung burden) of ^{144}Ce . This point is further illustrated in Table III, which summarizes the number of cases of primary neoplasia by organ of origin and radiation dose pattern. Detailed results have previously been published on several of the studies [9-15]. Space limitations preclude a detailed presentation of mortality data. Suffice it to note, however, that most of the dogs within the studies are more than 1500 days post-inhalation exposure, with some over 3000 days post-inhalation exposure, and most of the deaths due to neoplasia have been observed in animals with higher-level exposures. Such exposures have generally resulted in doses calculated in tens of thousands of rads to the tissues where the neoplasms have been observed to originate.

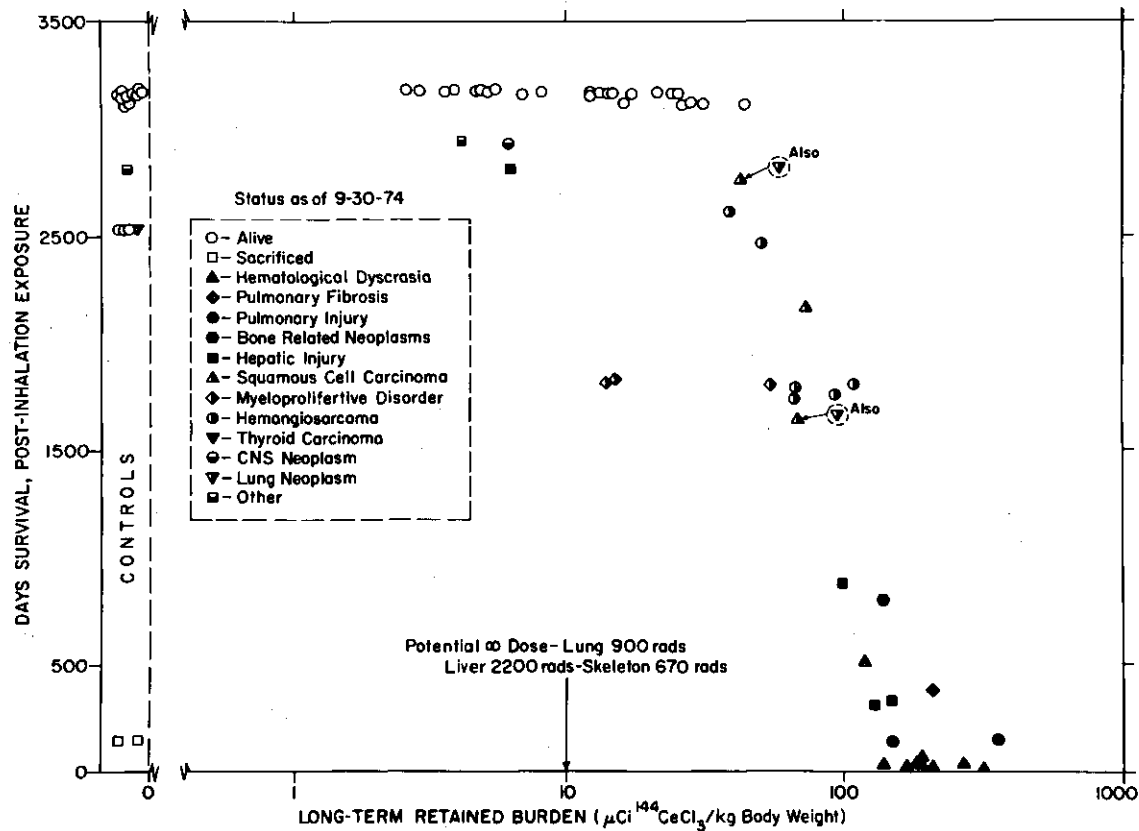


FIG. 3. Survival of beagles that inhaled $^{144}\text{CeCl}_3$ (status as of 9/30/74).

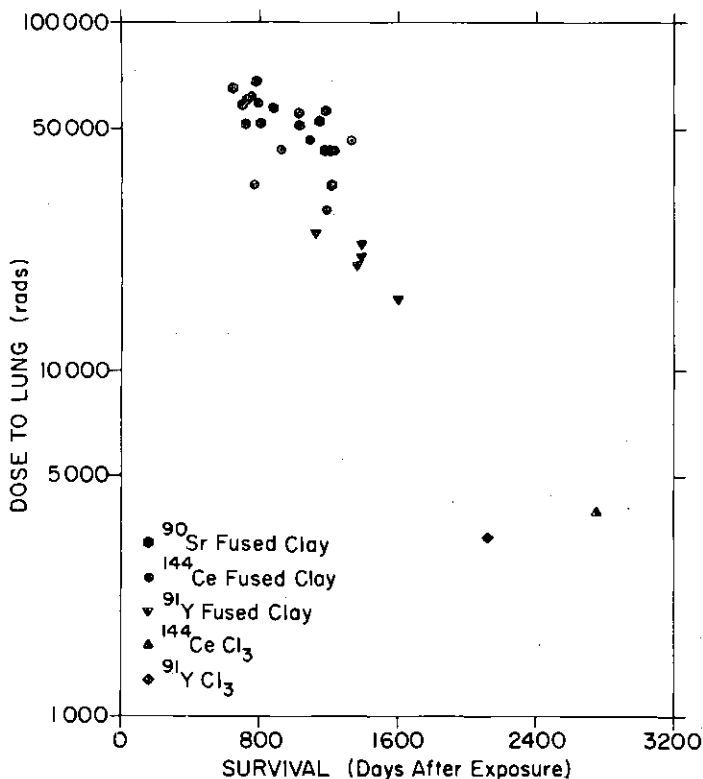


FIG. 4. Survival times and radiation dose to lung for dogs dying with primary lung neoplasms after inhalation of β -emitting radionuclides.

A total of 30 primary lung neoplasms have been observed to date. The survival time and the dose to lung for each dog with a neoplasm is shown in Figure 4. Most of the primary pulmonary neoplasms seen in dogs have been hemangiosarcomas. In addition, a few other malignant pulmonary neoplasms have been observed including bronchiolo-alveolar carcinoma, bronchiogenic adenocarcinoma, epidermoid carcinoma and fibrosarcoma. These have frequently been found in animals that also had a hemangiosarcoma.

Mouse Studies

The whole-body retention patterns of ^{144}Ce in mice that received either a single exposure to yield an initial lung burden of $1.0\ \mu\text{Ci}$ or repeated exposures to re-establish lung burdens of about $1.0\ \mu\text{Ci}$ are shown in Figure 5. Within a week after inhalation exposure, essentially all of the retained ^{144}Ce was found in the lung. Qualitatively similar patterns of whole-body and lung

Table III
Primary Malignant Neoplasms by Organ of Origin Related to Radiation Dose Pattern
from Inhaled Radioactive Aerosols (Status as of 7/1/75)

Radionuclide and Form	Number of Exposed Dogs	Lung		Skeleton**		Liver		Other Organs	Total
		Significant Dose	Number of Neoplasms	Significant Dose	Number of Neoplasms	Significant Dose	Number of Neoplasms	Number of Neoplasms	
$^{90}\text{SrCl}_2$	66		0	++++	34		0	3	37
$^{91}\text{YCl}_3$	46	++	1	++	2	+	0	2	5
$^{144}\text{CeCl}_3$	55	+++	1	+++	5	++++	5	4	15
^{90}Y in FAP*	89	++++	0		0		0	0	0
^{91}Y in FAP	96	++++	5		0		0	0	5
^{144}Ce in FAP	111	++++	8	+	2	+	0	4	14
^{90}Sr in FAP	106	++++	14	+	0		0	2	16
Controls (Composite)	111		0		0		0	2	2

*FAP - in Aluminosilicate Particles

**Bone or Bone Associated

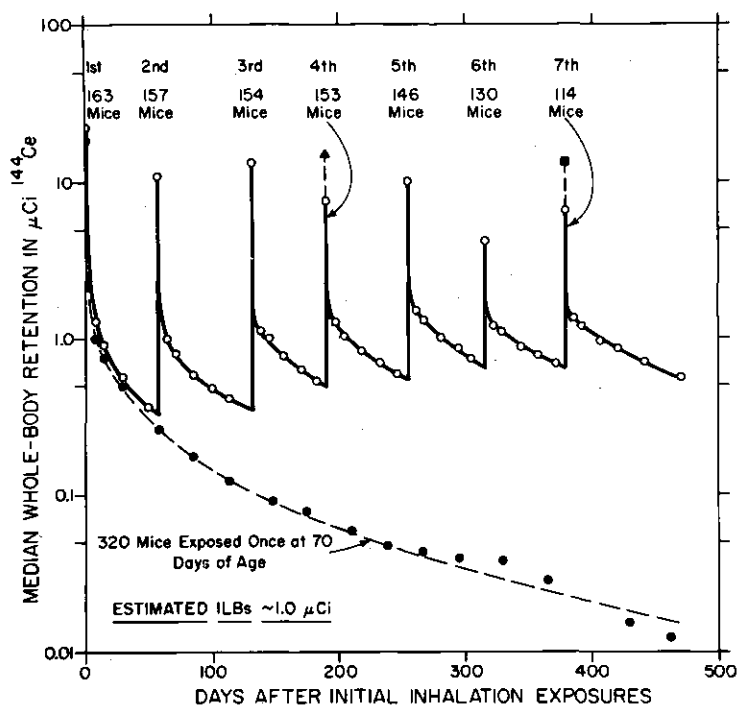


FIG. 5. Whole-body retention of ^{144}Ce in mice exposed once when at 70 days of age or repeatedly exposed to re-establish initial lung burdens of $1.0 \mu\text{Ci}$.

retention were observed in mice that received either higher or lower levels of exposure.

Since the main mouse study is still in progress, only preliminary results can be reported. Data obtained to date indicate only a small degree of life shortening in mice receiving single exposures to achieve an ILB of 0.2 or $1.0 \mu\text{Ci } ^{144}\text{Ce}$ and pronounced life shortening in mice receiving a single exposure to achieve an ILB of $4.5 \mu\text{Ci}$. Mice repeatedly exposed to sustain a lung burden of about $0.2 \mu\text{Ci}$ had a small degree of life shortening while lifespan was shortened by approximately one-third in mice repeatedly exposed to sustain a lung burden of about $1.0 \mu\text{Ci}$. Mice repeatedly exposed to sustain a lung burden of about $4.5 \mu\text{Ci}$ had a marked shortening of lifespan.

Histopathologic examinations are still under way on the tissues from the mice. In those examined to date, over a dozen pulmonary adenomas or carcinomas have been observed with about equal numbers of each. With the exception of an adenoma in a mouse exposed seven times to stable CeO_2 , all the neoplasms have been observed in mice repeatedly exposed to re-establish ^{144}Ce lung burdens of about 0.2 or $1.0 \mu\text{Ci}$.

DISCUSSION

Deaths observed at early time periods (< 1 yr post-inhalation exposure) were in the animals that had the highest lung burdens and consequently the highest initial dose rates. In these cases, the deaths were generally due either to radiation pneumonitis and pulmonary fibrosis or hematologic dyscrasias related to marrow aplasia dependent upon the relative dose to lung or bone marrow. The effectiveness per rad for producing early deaths due to radiation pneumonitis and pulmonary fibrosis was related to the pattern in which dose rate was delivered [16]. For materials in fused aluminosilicate particles deposited in lung, the effectiveness of ^{90}Sr and ^{144}Ce was lower than that for ^{91}Y , which was lower than for ^{90}Y . For the ^{90}Sr and ^{144}Ce , the initial dose rate was moderate but protracted, whereas for the ^{91}Y and ^{90}Y the dose rate to lung was initially higher but decreased more rapidly. The median survival time for animals dying early was shortest for ^{90}Y and ^{91}Y and longest for ^{144}Ce and ^{90}Sr . The effectiveness of the high dose rates was assumed to be due to the rate of tissue disruption exceeding the repair capacity of the tissue. At lower, protracted doses the repair capabilities of the tissue were sufficient to permit longer survival times.

When the initial dose rate was sufficiently low, deaths due to radiation pneumonitis and pulmonary fibrosis or marrow aplasia were avoided. In the case of animals exposed to ^{90}Y and ^{91}Y , the dose rate decreased sufficiently rapidly so that total doses accumulated by long-term survivors were generally less than 25 000 rads to lung and 3000 rads to skeleton for ^{91}Y and less than 10 000 rads to lung for ^{90}Y . These doses have been insufficient to produce many neoplasms during the first five years post-inhalation exposure (Table III). In contrast, with ^{144}Ce and ^{90}Sr the protracted dose rates to lung, liver and skeleton have resulted in high cumulative doses to these organs. Further, many neoplasms have been observed in these organs (Table III). With the exception of ^{90}Y in fused aluminosilicate particles from Table III, it is readily apparent that the number and organ distribution of neoplasms is strongly related to the relative dose received by the different organs. At this relatively early date in these long-term studies, it is too early to comment on the relative effectiveness of similar beta-radiation doses delivered to different organs.

The information presented in Figure 4 indicates that essentially all of the primary lung neoplasms observed to date in the dogs exposed to beta-emitting radionuclides in fused aluminosilicate particles have been associated with ^{144}Ce and ^{90}Sr . These have been the dose patterns that have yielded

protracted beta irradiation of lung and associated high cumulative doses. It is especially noteworthy that no primary lung neoplasms have yet been observed in 51 dogs that received 1300 to 10 000 rads to lung in a brief period of time following inhalation of ^{90}Y in fused aluminosilicate particles. Because the end point of primary interest, lung neoplasia, has not yet been observed in dogs exposed to ^{144}Ce or ^{90}Sr in fused aluminosilicate particles at doses comparable to those received by the dogs that inhaled ^{90}Y or ^{91}Y in fused aluminosilicate particles, it is not possible to determine the relative importance of total dose versus dose protraction. Conceivably the lack of primary lung neoplasms in dogs exposed to ^{90}Y and ^{91}Y in fused aluminosilicate particles is related to the lower total doses they have received. On the other hand, it is possible that these dose patterns with high dose rate exposures that decreased rapidly are not as effective as protracted exposure which results in continuing tissue damage. An additional factor may be latent period, the duration of which may be related to the dose pattern. Since dogs have been exposed to levels of ^{90}Y , ^{91}Y , ^{144}Ce or ^{90}Sr in fused aluminosilicate particles resulting in cumulative lung doses for each study in the range of 5000 to 10 000 rads, it will ultimately be possible to examine the relative

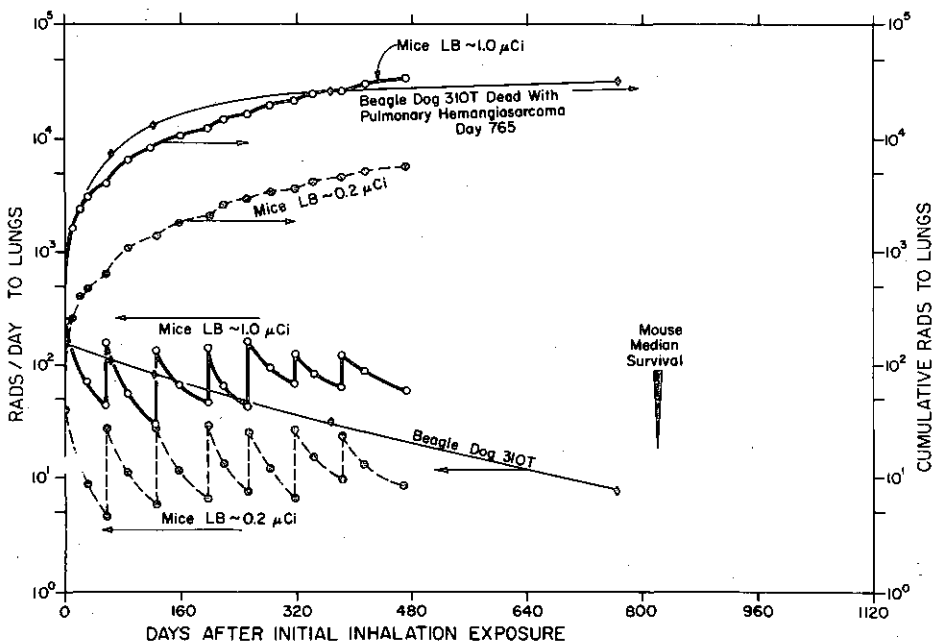


FIG. 6. Radiation dose rate and cumulative dose rate to lung of beagle exposed once to ^{144}Ce in fused aluminosilicate particles and mice exposed repeatedly to $^{144}\text{CeO}_2$ particles.

importance of the manner in which dose rate decreases versus total dose on the frequency, time distribution of appearance and type of lung neoplasms induced.

The repeated exposure regime for the mice, as expected, yielded a dose pattern similar to that observed following a single brief exposure of dogs (Fig. 6). The cumulative dose to the lung of mice maintained with lung burdens of about 1.0 μCi is nearly identical to that of dog 310T that died with a primary hemangiosarcoma of the lung 765 days after inhaling ^{144}Ce in fused aluminosilicate particles.

Of special interest is the finding that mice exposed repeatedly to $^{144}\text{CeO}_2$ developed primary lung neoplasms at cumulative lung doses similar to those that produced primary lung neoplasms in the dog. Thus it would appear that the two species respond in a qualitatively similar manner to chronic beta-radiation to lung when the dose pattern is similar. The extent to which there are quantitative differences in the response of the two species when the dose patterns are similar should be apparent when the present studies are completed. A histopathologic review of all of the material from the completed studies should provide an assessment of the extent to which there are species differences in the types of neoplasms induced by similar radiation dose patterns.

ACKNOWLEDGEMENTS

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DISCUSSION

W.H. ELLETT: Your preliminary results indicate that protracted irradiation is more radiocarcinogenic than acute exposures. This is in contrast to the results of in-vitro experiments where cell death is used as an endpoint, for these results have often been interpreted as indicating reduced effects from lower dose rates. Do you think that the observed increase in radiocarcinogenesis in the lung from protracted radiation exposure will prove to be generally true for other organs subject to prolonged exposure?

R.O. McCLELLAN: As the present studies are still in progress, it is not possible to state categorically that protracted exposure is more carcinogenic than briefer exposures. One difficulty in interpreting the incomplete studies is that the brief high-dose exposures are more effective for producing early effects, including death, than the protracted exposures. As a result, the animals which receive the briefer exposures, survive, and then are available for long-term observation, have lower cumulative doses than the protracted-exposure animals which are so available. It is the latter animals which are currently developing many malignant primary lung neoplasms. It is important to recognize that they have greater cumulative doses than the long-term survivors that received brief exposures. The apparent greater radiocarcinogenicity may be related to this higher dose and the duration of our observations. It will be of interest to observe, over the total lifespan of the animals, the comparative carcinogenic response of those which received 5000 to 10 000 rad with a spectrum of dose patterns (from ^{90}Y in fused clay with the dose delivered in a few days to ^{90}Sr in fused clay with the dose delivered over a long period of time).

R.J.M. FRY: Speaking about extrapolation between species, you included lifespan and latent period in the list of factors to be taken into account. Could you please say how you feel about these two factors being used in extrapolation?

R.O. McCLELLAN: I am uncertain as to how we can take lifespan and latent period precisely into account in extrapolating experimental animal data to man. Nonetheless I am certain that we must consider both factors. Although we have generally assumed a correlation between lifespan and latent period, I believe our knowledge of these factors and their relationship with each other is insufficient; we should therefore be cautious and consider them independently. Do you have information on the important question of the relationship between lifespan and latent period?

R.J.M. FRY: There is a limited amount of information of interest on sarcomas from an experiment started by Austen Brues some years ago.

Disks containing $^{90}\text{Sr}/\text{Y}$ were put into the rat, which has a maximum life-span of about three years, the dog with a maximum lifespan of about fourteen years and Peromyscus leucopus with a maximum lifespan of seven to eight years. The species of interest was P. leucopus and the latent period, at least of the tumours appearing early, was the same as for the rat.

COMPARISON OF CARCINOGENICITY OF ^{131}I AND ^{125}I IN THYROID GLAND OF THE RAT

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Abstract

COMPARISON OF CARCINOGENICITY OF ^{131}I AND ^{125}I IN THYROID GLAND OF THE RAT.

To determine whether there was a difference in the carcinogenicity of ^{131}I and ^{125}I for the thyroid gland, groups of 40 rats on a low-iodine diet were injected with various doses of one of the nuclides. The doses of ^{131}I injected were 125, 50, 20, 8 and 3.2 μCi ; doses of ^{125}I were four times higher. The surviving rats were killed two years after injection and autopsies with complete histopathological examinations were performed. The results of survival and thyroid function studies revealed that the dose ranges in μCi for the two nuclides could not be directly compared and that each dose of ^{125}I could best be compared with a 10- to 25-fold lower dose of ^{131}I . The morphological findings in the follicular epithelium of the thyroid gland showed that the peak of induction of hyperplasia, cysts and tumours by ^{125}I occurred in the group injected with 80 μCi , whereas comparable changes after ^{131}I administration were observed in the group injected with the lowest dose (3.2 μCi). The peak of tumour induction by ^{125}I in the parafollicular epithelium was found in the group injected with 32 μCi ; the peak with ^{131}I was probably missed, since the comparable dose for this nuclide was below the range of doses injected. The highest incidence of tumours of these parafollicular cells was 2.5 times the spontaneous incidence in control rats. However, extrapolation of these results to man seems unreliable, since the spontaneous incidence of tumours of the parafollicular cells is much higher in rats than in man. It may be concluded that ^{125}I does not induce more tumours of the follicular cells than does ^{131}I as has been suggested by others.

1. INTRODUCTION

The radioisotopic treatment of thyrotoxicosis is generally performed with ^{131}I . Unfortunately, ^{131}I therapy leads to hypothyroidism in a large proportion of these patients. Because of this, the possibly preferable use of ^{125}I instead of ^{131}I was studied in a number of clinical trials during the last few years [1 - 11]. These studies were based on the hypothesis of Greig et al. [1] that results could be improved by the use of this isotope. Greig hypothesized that, when concentrated in a follicle, ^{125}I would, by the

emission of soft electrons, irradiate the apices of the follicular cells more intensively than the nuclei. Since the hormonogenesis occurs at the apices of the follicular cells, the hormone-producing sites in these cells might be selectively damaged with minimal nuclear damage. On the basis of this difference in dose distribution, it was expected that therapeutic doses of ^{125}I might control thyrotoxicosis with less postirradiation hypothyroidism, secondary to cell death.

However, studies performed by Gross et al. [12] demonstrated the occurrence of follicular tumours in the thyroid glands of two out of 25 rats given $25\text{ }\mu\text{Ci}$ of ^{125}I , whereas no tumours were observed in a similar group of rats after $25\text{ }\mu\text{Ci}$ of ^{131}I . Since a certain dose in μCi of ^{131}I will damage the thyroid gland more severely than a similar dose of ^{125}I , the carcinogenic effect cannot be compared at similar μCi doses of both nuclides.

For that reason, a more systematic study of the comparative carcinogenic effect of these isotopes was considered imperative. Our study included the comparison of the carcinogenic effects after injection of various doses of both nuclides into rats which had been kept on a low-iodine diet to increase the height of the follicular cells. As has been observed earlier [13], this makes the distribution of radiation doses over the cells comparable to that in Graves' disease. The carcinogenicity of isotopes was compared after doses of both isotopes selected to cause a similar loss of thyroid function. The loss in thyroid function was determined by the depression of the radioactive iodine uptake in the thyroid and reduction in thyroid hormone production.

2. MATERIALS AND METHODS

2.1. Rats and administration of isotopes

Female BN/Bi rats, 8-12 weeks of age, were injected i.p. with various doses of ^{131}I and ^{125}I (carrier-free NaI from the Radiochemical Centre, Amersham, United Kingdom). The rats were fed a low-iodine diet (Hope Farms, iodine content $0.04\text{ }\mu\text{g/g}$) and distilled water from two weeks before injection until two weeks afterwards. Normally, the rats were kept on a standard diet which contained $0.19\text{ }\mu\text{g}$ iodine/g. This study was carried

TABLE I. INJECTION SCHEME OF ^{131}I AND ^{125}I IN μCi

^{131}I		^{125}I	
planned	administered* $\bar{x} \pm \text{S.E.}$	planned	administered* $\bar{x} \pm \text{S.E.}$
125	119.4 ± 7.2	500	513.0 ± 13.3
50	48.6 ± 2.5	200	205.4 ± 9.4
20	19.6 ± 1.1	80	83.0 ± 1.9
8	7.9 ± 0.4	32	33.5 ± 1.2
3.2	3.2 ± 0.2	12.8	13.3 ± 0.3

*administered in five replicate experiments (5 x 8 rats per dose group)

out in five replicate experiments, with eight rats per dose group. The radioactivity of the injected doses was verified by comparison with calibrated standard solutions for both nuclides (Amersham). The thyroid uptake was $51 \pm 3\%$ ($\bar{x} \pm S.E.$), whereas the biological half-life was 3.5 ± 0.1 days, except for the highest two doses of ^{131}I ($125 \mu\text{Ci}$ and $50 \mu\text{Ci}$). These biological half-lives were 1.9 days and 2.9 days, respectively. The deviations of the injected doses from the planned doses are presented in Table I. In the first of the replicate studies, half of the surviving rats were killed and necropsied at 18 months after the injection of the isotopes. The remainder of the rats in this study and all rats in the subsequent replicates were killed and studied at two years after injection.

2.2. Thyroid function studies

The thyroid function was studied in the rats surviving at two years after injection. Three parameters for thyroid function were studied: (1) the iodine tracer uptake in the thyroid gland; (2) the thyroxine (T_4) levels in serum; and (3) in some rats, the thyroid-stimulating hormone (TSH) levels in serum. The iodine uptake in the thyroid gland was determined at 20-28 hours after i.p. injection of $1 \mu\text{Ci}$ of ^{131}I . This time interval was chosen because the iodine uptake in the thyroid showed a plateau between 20 and 29 hours after injection. To determine the percentage of ^{131}I uptake, the rats were killed with an overdose of ether and the thyroid gland with the trachea was removed and fixed in 2 mlitre 4% buffered formalin. The radioactivity in the thyroid gland was counted in a γ -well-type scintillation counter and the uptake percentage was calculated by comparison with the injected radioactivity.

The T_4 level in serum was determined by radioimmunoassay [14] as was the TSH level (kindly performed by G. Morreale de Escobar, Madrid).

2.3. Histological examination

All rats, whether killed at the ages stated in Section 2.1, found dead, or killed when moribund, were subjected to a complete necropsy. The necropsy and histological techniques for the BN/Bi rat were identical to those described for the WAG/Rij rat [15]. The sections were routinely stained with haematoxylin-phloxin-saffran. The thyroid with trachea was divided in half prior to embedding. The thyroids were sectioned semi-serially, i.e. one sample from each of ten sections was taken for staining and examination by light microscopy. The pathological findings were reported as lesions of the follicular cells and of the parafollicular cells. Abnormal morphology of the follicular cells was classified as either hyperplasia, follicular cysts or tumours, which included both adenomas and carcinomas. The lesions of the parafollicular cells were divided into hyperplasia and medullary thyroid carcinoma.

3. RESULTS

3.1. Survival at two years after injection of isotopes

The determination of thyroid function and the histopathological examinations were carried out at two years after injection of the isotopes.

TABLE II. PERCENTAGE OF RATS SURVIVING AT TWO YEARS AFTER SINGLE DOSES OF ^{131}I AND ^{125}I

dose of ^{131}I in μCi	percent survivors	nr.survivors/ nr.injected	dose of ^{125}I in μCi	percent survivors	nr.survivors/ nr.injected
125	9	3 / 35*	500	59	20 / 34
50	20	7 / 35*	200	61	22 / 36
20	78	28 / 36	80	78	28 / 36
8	75	27 / 36	32	72	26 / 36
3.2	89	32 / 36	12.8	85	28 / 37
control	78	52 / 67			

* significantly different from control

 $p < 0.05$ (χ^2 test)TABLE III. EFFECT OF SINGLE DOSES OF ^{131}I AND ^{125}I ON IODINE UPTAKE OF RAT THYROID TWO YEARS AFTER INJECTION

dose of ^{131}I in μCi	percent uptake $\bar{x} \pm \text{S.E.}$	n	dose of ^{125}I in μCi	percent uptake $\bar{x} \pm \text{S.E.}$	n
125	0.6* \pm 0.1	3	500	29.1* \pm 2.7	20
50	22.8* \pm 2.8	7	200	33.0* \pm 1.6	22
20	32.0* \pm 1.4	28	80	38.6* \pm 1.7	28
8	35.7* \pm 1.2	27	32	42.0 \pm 2.0	26
3.2	40.1 \pm 2.2	32	12.8	45.6 \pm 2.1	28
control	44.1 \pm 1.5	52			

* significantly different from control

 $p < 0.05$ (Student's t-test)

However, a proportion of rats had already died by that time. The fraction of the rats surviving at two years after injection is presented in Table II as percentage of survivors and as total number of injected rats that survived. A significantly decreased fraction of survivors is observed in the highest two dose groups of ^{131}I . In these dose groups, death before the time of sacrifice was mostly associated with extensive tumours of the hypophysis. These tumours were often greater than 0.5 cm in diameter, causing marked cerebral compression. On the basis of light microscopic appearance, these tumours were classified as chromophobe adenomas. No significant difference in survival, as compared to controls, was observed in the other dose groups of both ^{131}I and ^{125}I .

TABLE IV. EFFECT OF SINGLE DOSES OF ^{131}I AND ^{125}I ON T_4 LEVELS IN SERUM TWO YEARS AFTER INJECTION

dose of ^{131}I in μCi	T_4 μg per 100 ml $\bar{x} \pm \text{S.E.}$	n	dose of ^{125}I in μCi	T_4 μg per 100 ml $\bar{x} \pm \text{S.E.}$	n
125	0.23* \pm 0.1	3	500	1.7* \pm 0.2	17
50	1.4* \pm 0.3	6	200	2.6* \pm 0.2	22
20	2.2* \pm 0.1	27	80	2.9* \pm 0.1	28
8	2.6* \pm 0.1	27	32	3.1 \pm 0.1	25
3.2	2.8* \pm 0.1	27	12.8	3.3 \pm 0.1	28
control	3.4 \pm 0.1	42			

* significantly different from control

 $p < 0.05$ (Student's t-test)TABLE V. EFFECT OF SINGLE DOSES OF ^{131}I AND ^{125}I ON TSH LEVELS IN SERUM TWO YEARS AFTER INJECTION

dose of ^{131}I in μCi	TSH μ Units/ml	dose of ^{125}I in μCi	TSH μ Units/ml
125	19 - 6	500	2.6 - 6.8 - 15.3 27.3 - >30
50	1.4 - 8.5 - 10.3 - 13.9		
3.2	0.2 (5x) - 0.9		
control	<0.2 (5x) - 2.1		

3.2. Iodine uptake

Table III shows the uptake percentage of iodine in the thyroid gland. It is evident that almost no uptake is observed in the highest dose group of ^{131}I , but this result is based on only three surviving rats. For both isotopes there is a clear relation between treatment dose and isotope uptake, the decrease being more severe after higher doses.

3.3. T_4 levels

The T_4 levels, expressed as $\mu\text{g}/100$ mlitre serum, are presented in Table IV. A significantly decreased level of T_4 was found in all dose groups of ^{131}I and in the highest three dose groups of ^{125}I .

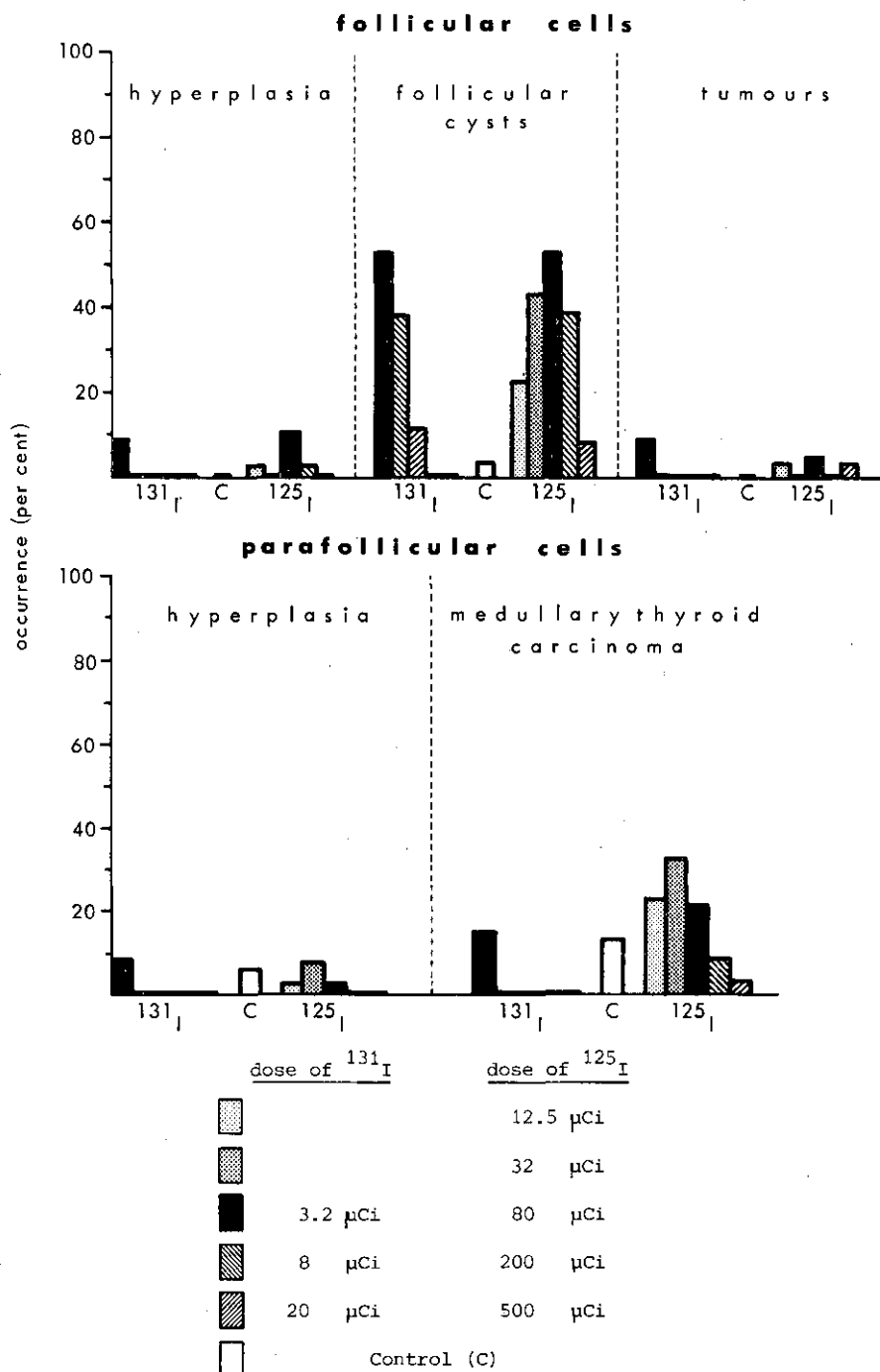


FIG.1. Effect of single doses of ^{131}I and ^{125}I on the occurrence of thyroid cell lesions at two years after injection. No lesions of the indicated type were observed in the small remnants of the thyroid gland after 50 μCi and 125 μCi of ^{131}I . On the basis of survival and thyroid function studies it appeared that the ratio of effectiveness for $^{125}\text{I}/^{131}\text{I} \sim 10-25$.

3.4. TSH levels

In some dose groups, it was investigated whether the status of the thyroid function, as determined by the iodine uptake and the T_4 levels in serum, was also reflected in the level of thyroid-stimulating hormone in the serum. The results are presented in Table V. An increased TSH level was found in the highest dose group of ^{125}I and a similar increase in the highest two dose groups of ^{131}I , whereas no increase is observed in the lowest dose group of ^{131}I .

3.5. Histopathological examination

Almost no thyroid tissue could be observed in the rats treated with the highest dose (125 μCi) of ^{131}I . A marked atrophy of the thyroid gland was observed in the 50 μCi of ^{131}I group and in the highest dose (500 μCi) group of ^{125}I , whereas the degree of atrophy decreased with a decrease in dose of both isotopes. The thyroid lesions at two years after injection are presented in Fig. 1 and Table VI. A slight increase in incidence of hyperplasia and tumours of the follicular cells was observed after the lowest dose of ^{131}I and some doses of ^{125}I . However, the incidence of follicular cysts was markedly increased. The highest incidence (53%) was observed after 3.2 μCi of ^{131}I and 80 μCi of ^{125}I . No difference was observed in the occurrence of hyperplasia of the parafollicular cells. However, the incidence of tumours of these cells (medullary thyroid carcinoma) was increased in the groups injected with 12.8, 32 and 80 μCi ^{125}I . This increase was significant only in the group injected with 32 μCi ^{125}I .

4. DISCUSSION

To compare the carcinogenicity of ^{131}I and ^{125}I , doses of each isotope should be chosen which achieve the same reduction in thyroid function. To be clinically meaningful this reduction should be of a similar degree to that desired in the treatment of thyrotoxicosis. Since the degree of reduction in thyroid function in the rat comparable to that in man is not known, a range of equally effective doses of both isotopes must be compared in order to include at least one dose which is clinically relevant. We have included this in our experimental design (Table VII). It appeared that the ratio of effectiveness for $^{125}\text{I}/^{131}\text{I}$ is 10 - 25. Thus, the carcinogenicity after certain doses of ^{125}I could best be compared with doses expressed in μCi , of ^{131}I that are lower by a factor of 10 to 25. A similar factor in relative effectiveness between the two isotopes was observed by Jongejan and van Putten in mice and rats [13].

If the occurrence of follicular cell lesions is considered, it is evident that only a slight increase in hyperplasia and tumours was found with either isotope. However, a highly significant induction of follicular cysts was observed. The peak of cyst induction was found in the group injected with 80 μCi of ^{125}I , whereas a similar frequency of cysts was observed in the comparable dose groups of ^{131}I (3.2 μCi and 8 μCi). Although hyperplasia, but not cyst formation, is considered a premalignant lesion in experimentally induced thyroid cancer [16], the distribution of the few tumours in the same

TABLE VI. EFFECT OF SINGLE DOSES OF ^{131}I AND ^{125}I ON THE OCCURRENCE OF THYROID LESIONS TWO YEARS AFTER INJECTION

dose in μCi	total number of rats injected	Follicular cells			Para-follicular cells	
		Hyperplasia nr.of rats (%)	Follicular cysts nr.of rats (%)	Tumours nr.of rats (%)	Hyperplasia nr.of rats (%)	Medullary Thyroid Carcinoma nr.of rats (%)
<u>¹³¹I</u>						
125	35	- (-)	- (-)	- (-)	- (-)	- (-)
50	35	- (-)	- (-)	- (-)	- (-)	- (-)
20	37	- (-)	4 (11)	- (-)	- (-)	- (-)
8	34	- (-)	13 (38 [*])	- (-)	- (-)	- (-)
3.2	34	3 (9)	18 (53 [*])	3 (9)	3 (9)	5 (15)
<u>¹²⁵I</u>						
500	36	- (-)	3 (8)	1 (3)	- (-)	1 (3)
200	36	1 (3)	14 (39 [*])	- (-)	- (-)	3 (8)
80	38	4 (11 [*])	20 (53 [*])	2 (5)	1 (3)	8 (21)
32	37	- (-)	16 (43 [*])	- (-)	3 (8)	12 (32 [*])
12.8	35	1 (3)	8 (23 [*])	1 (3)	1 (3)	8 (23)
control	69	- (-)	3 (4)	- (-)	4 (6)	9 (13)

* significantly different from control

 $p < 0.05$ (χ^2 test)

TABLE VII. COMPARABILITY OF ISOTOPE EFFECT AMONG DIFFERENT GROUPS

		lowest dose of ^{131}I (3.2 μCi) is comparable with
Survival	(table II)	80 μCi ^{125}I
Iodine uptake	(table III)	32 - 80 μCi ^{125}I
T_4 levels	(table IV)	32 μCi ^{125}I

dosage ranges in which higher frequencies of cysts and hyperplasia are noted could be cited in favour of a relationship between these abnormalities.

An increased occurrence of tumours of the parafollicular cells was observed in some dose groups injected with ^{125}I . However, this increase was significant only after 32 μCi of ^{125}I . Since these parafollicular cells are located interstitially, they receive less irradiation from the short-range soft electrons of ^{125}I than do the follicular cells. Because of this, the difference in radiation dose distribution per μCi between the two isotopes will be more than the factor of 10 to 25 that was observed for follicular cell function. Since the highest incidence of this medullary thyroid carcinoma was observed in the group injected with 32 μCi ^{125}I , the comparable dose of ^{131}I will probably be below the range of doses injected. Some confirmation for this assumption is obtained by the increased incidence of this parafollicular tumour after a single dose of 5 μCi of ^{131}I in a study by Lindsay [17]. Five μCi is comparable to a dose of about 1 μCi under the conditions of our study, since a higher uptake of the injected ^{131}I dose is obtained in our study due to the low-iodine diet before injection. Furthermore, single doses of 1 μCi and 10 - 40 μCi of ^{131}I did not significantly alter the natural incidence [18 - 20], whereas doses of 100 μCi and higher prevented the growth of these tumours. In our study, lower doses (8 μCi and higher) prevented their growth. This prevention may be considered a consequence of the elimination of potentially malignant cells, a well-known mechanism responsible for a decrease in tumour incidence after high doses of radiation [21].

If the induction of follicular and parafollicular lesions is compared, it is clear that doses of 8 and 20 μCi of ^{131}I prevented the growth of the naturally occurring parafollicular tumours, whereas similar doses induced cysts of the follicular cells. Since ^{131}I will irradiate the thyroid gland rather homogeneously, the radiation dose to both cell types will be similar. For that reason, the difference in radiation response might indicate a difference in intrinsic sensitivity to radiation between both cell types in rats.

The high incidence of naturally-occurring tumours of the parafollicular cells seen in this study is also observed in a number of other strains of rats [17, 20]. It has been shown that these lesions increase with age as well as with iodine deficiency [22]. The high incidence in rats is in contrast to the low incidence in man [23, 24]. The apparent increase in medullary thyroid carcinoma after exposure to radiation confirms the observation of Lindsay et al. [20].

A similar relationship to radiation has not been observed in man. A report of the Co-operative Thyrotoxicosis Therapy Follow-up study [25] indicated no difference in the incidence of cancer in patients treated for hyperthyroidism either by radioiodine therapy or by operation. In 19186 patients treated with ^{131}I , 19 malignant tumours were observed. Of these one solid carcinoma might be considered as a medullary thyroid carcinoma. Since the doses of ^{131}I used for the treatment of hyperthyroidism in man are comparable to the higher range of doses in our studies, the lower incidence observed in our rats after 20 to 50 μCi agrees with the low incidence of tumours of the thyroid in man. The medullary thyroid carcinoma in the rat has many properties in common with the human medullary thyroid carcinoma [26, 27]. However, Lindsay et al. [16, 17] made a very clear distinction between these naturally-occurring TSH-independent medullary thyroid carcinomas and those tumours of the follicular cells, induced by radiation or by goitrogens. The high spontaneous incidence in our rats – whether induced by the relatively low iodine intake or not [22] – suggests that the incidence of rat medullary thyroid tumours cannot predict for the situation in man.

In contrast, a clear similarity exists between the increased induction by ^{131}I of tumours of the follicular cells of the thyroid at early ages in animals and man [16]. For that reason the comparison between ^{131}I and ^{125}I concerning tumour induction of the follicular cells in rats can be extrapolated to man with some confidence.

Finally, it may be concluded that our findings concerning the frequency of tumours of the follicular cells are compatible with the results obtained by Gross et al. [12], since we found a similar tumour incidence in some of our groups. However, our more extensive data do not support the conclusion that ^{125}I is more carcinogenic than ^{131}I .

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DISCUSSION

Helen WOODARD: The paper gives a clear demonstration of the relation between the administered dose of the two isotopes and the magnitude of various effects on the thyroid. Were calculations made of the rad doses in the thyroid glands and also in the pituitaries, which in the rat are close enough to the thyroid to receive a significant dose from the more penetrating irradiations from nuclides deposited in the thyroid? In comparing rats and man, it must also be remembered that there is considerably more "wasted radiation" in the thyroid of the rat than in that of man.

J. de RUITER: No calculations were performed of the radiation dose in the thyroid gland since, especially in the case of ^{125}I , it is rather arbitrary. The soft short-range electrons of ^{125}I will be absorbed within the first few μm from the colloid. For that reason, the radiation dose over the follicular cells is rather inhomogeneous and the dose to the nuclei will be several times lower than the dose to the apical parts of the cells. This is in contrast to the fairly homogeneous β -radiation of ^{131}I over the cells. It is therefore difficult to compare the radiation dose for the two isotopes at the cell level. Secondly, the iodine is distributed inhomogeneously over the thyroid gland, which contains active and inactive follicles. The active follicles will accumulate some radioactive iodine, whereas the inactive ones will accumulate less or none at all. In the case of ^{131}I , the cells of an inactive follicle may receive the radiation of the surrounding active follicles, but this is not true for ^{125}I . So we concluded that calculation of the mean radiation dose to the thyroid gland would not provide additional information about cells receiving the radiation dose which could be responsible for malignant

TABLE A

EFFECT OF SINGLE DOSES OF ^{131}I AND ^{125}I ON THE TUMOUR INCIDENCE
IN HYPOPHYSIS AND ADRENAL

dose in μCi	Hypophysis		Adrenal	
	nr. tumours/ nr. examined	per cent	nr. tumours/ nr. examined	per cent
^{131}I				
125	29/35	83*	22/25	88*
50	26/35	74*	17/25	68*
20	13/37	35*	7/25	28
8	9/34	26	2/26	8
3.2	5/34	15	1/28	4
^{125}I				
500	18/36	50*	10/26	38
200	5/36	14	3/25	12
80	12/38	32*	3/25	12
32	3/37	8	3/26	12
12.8	6/35	17	4/27	15
Control	9/69	13	11/50	22

* $p < 0.05$ (Student's t-test).

transformation. I agree with you as regards the differences in "wasted radiation" between the thyroid glands of rats and man. Therefore the $^{125}\text{I}/^{131}\text{I}$ effectiveness ratio, which is of the order of 10-25 in our rats, cannot be extrapolated to man.

K. SHIMAOKA: We have been working with transplantable thyroid tumours induced in Fischer rats with ^{131}I . Medullary carcinoma is rarely seen in this series. Have you investigated whether your medullary carcinomas produce thyrocalcitonine or amyloid? Have you tried transplantation of your thyroid tumours?

J. de RUITER: No, on this occasion we did not study the production of calcitonine of the medullary thyroid carcinoma. Transplantation of medullary thyroid carcinoma has been performed successfully for several passages.

R. J. M. FRY: In Fig. 1 you show the incidences of medullary carcinoma and hyperplasia in control rats. The incidence of carcinoma is greater than that of hyperplasia. Does this indicate that hyperplasia is not a pre-cancerous stage?

J. de RUITER: I cannot say whether this conclusion could be drawn from our findings.

K.H. CLIFTON: How old were your rats at the time of isotope injection, and could you please tell us more about the pituitary tumours which you observed?

J. de RUITER: Our rats were 8 - 12 weeks old at the time of injection of the isotopes. The incidence of pituitary tumours after the various doses of both isotopes can be seen from Table A. A highly significant increase in incidence is observed after the highest dose of both isotopes. The high incidence of pituitary tumours is accompanied by a high incidence of adrenal tumours.

K.H. CLIFTON: Thyrotropin-secreting pituitary tumours develop in mice after thyroidectomy but have not been reported in rats. It would be most interesting if such TSH-secreting tumours could be established in transplantation in rats.

ETUDE EXPERIMENTALE DE L'ACTION DE DEUX EMETTEURS BETA INHALES: LE CERIUM-144 ET LE CERIUM-141 Raccourcissement de la durée de vie et induction de cancers — Rôle de la dose — Influence de l'entraîneur

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Abstract-Résumé

EXPERIMENTAL STUDY OF ACTION OF INHALED CERIUM-144 AND CERIUM-141: LIFE SPAN REDUCTION AND CANCER INDUCTION — ROLE OF DOSE — INFLUENCE OF CARRIER.

Studies were undertaken to compare the toxic action of inhaled β - and α -emitters. About a hundred rats were contaminated by inhalation of acidic ^{144}Ce chloride. The initial activities ranged from 0.5 μCi to 10 μCi , so that the rats were subjected to doses of 500-5000 rads. It was observed that, depending on the dose, the life span of the animals was shortened and some ten lung cancers appeared in the animals which had received the highest doses. In another experiment, the activation product ^{141}Ce is inhaled in oxide or chloride form, the doses ranging from 50 rads to 2000 rads. Other animals inhaled stable cerium oxide. Lung cancers have already been observed in the animals which inhaled ^{141}Ce . The locations and histological types of the cancers induced by the three β -emitters are comparable with those observed after the inhalation of α -emitters.

ETUDE EXPERIMENTALE DE L'ACTION DE DEUX EMETTEURS BETA INHALES: LE CERIUM-144 ET LE CERIUM-141 — RACCOURCISSEMENT DE LA DUREE DE VIE ET INDUCTION DE CANCERS — ROLE DE LA DOSE — INFLUENCE DE L'ENTRAINEUR.

Des études ont été entreprises pour comparer l'action toxique des émetteurs bêta inhalés à celle des émetteurs alpha administrés par la même voie. Une centaine de rats ont été contaminés par inhalation de chlorure de cérium-144 acide. Les activités initiales déposées allaient de 0,5 à 10 microcuries, ce qui a soumis les animaux à des doses allant de 500 à 5000 rad. On a observé, suivant les doses, un raccourcissement de la durée de vie et l'apparition d'une dizaine de cancers du poumon chez les animaux ayant reçu les doses les plus élevées. Une autre expérience est en cours en utilisant du cérium-141, produit d'activation qui a été inhalé sous forme d'oxyde ou de chlorure. Les doses administrées allaient de 50 à 2000 rad. D'autres animaux ont inhalé de l'oxyde de cérium stable. On a déjà observé chez les animaux ayant inhalé du cérium-141 l'apparition de cancers du poumon. Les localisations et les types histologiques des cancers induits par les trois émetteurs bêta sont comparables à ceux observés après inhalation d'émetteurs alpha.

Depuis plusieurs années une expérimentation est en cours pour étudier l'action toxique d'émetteurs bêta inhalés chez le rat et la comparer à celle des émetteurs alpha administrés par la même voie.

Nous avons utilisé comme radioélément inhalé le cérium-144 sous forme de chlorure avec et sans entraîneur et le cérium-141 sous forme de chlorure et d'oxyde. Pour les chlorures les solutions de départ étaient à pH acide.

* Association CEA/Euratom.

** CEA, Département de protection.

Les expérimentations portent sur 250 animaux, 100 pour le cérium-144 et 150 pour le cérium-141.

Les activités pulmonaires initiales, mesurées par comptage global des animaux trois jours après l'inhalation, ont varié de 300 à 8000 nCi pour le cérium-144, ce qui a soumis les animaux à des doses allant de 200 à 4000 rad. Pour le cérium-141 les activités pulmonaires initiales allaient de 200 à 16 000 nCi, ce qui correspond à des doses de 30 à 2000 rad.

Les activités cumulées exprimées en nombre total de particules étaient de $20 \text{ à } 460 \times 10^9$ pour le cérium-144 et de $10 \text{ à } 860 \times 10^9$ pour le cérium-141.

Nous avons étudié l'influence des doses administrées au poumon sur la durée de vie et l'apparition des cancers du poumon. Nos techniques de contamination et de mesure de la radioactivité ont été décrites dans des publications antérieures [1, 2], ainsi que la méthode utilisée pour l'étude histologique du poumon [3].

On a observé, suivant les doses administrées, un raccourcissement de la durée de vie, dû surtout à des bronchites, des broncho-pneumonies et des lésions d'irradiation du poumon traduites par une pneumonie interstitielle.

Chez une partie des animaux, ayant une durée de vie plus grande que la durée moyenne du groupe, nous avons observé l'apparition de cancers du poumon.

Etant donné que l'expérimentation est encore en cours, les figures et les tableaux présentés ne donnent que des résultats provisoires.

Nous avons constaté qu'à l'exception de deux groupes d'animaux (auxquels avait été administré de l'oxyde de cérium-141 à faible dose), dont 80 et 90% ont disparu précocement par suite d'une broncho-pneumonie due à une épidémie générale, la durée de vie est en relation directe avec la dose administrée.

L'examen anatomo-pathologique a révélé l'existence de cancers pulmonaires. La figure 1 montre la mortalité moyenne par groupes d'animaux et la mortalité moyenne des rats cancéreux du même groupe en fonction de l'activité cumulée après inhalation de cérium-144 avec et sans entraîneur. On peut constater que la survie des animaux est d'autant plus longue que les doses administrées sont faibles.

On constate l'apparition de cancers du poumon à plus faible dose chez les animaux ayant inhalé du cérium-144 avec entraîneur, ce qui est dû à une diffusion plus lente [1]. La relation entre l'activité administrée et la durée de vie des animaux correspond aux observations faites après inhalation d'émetteurs alpha [4].

La figure 2 résume les résultats provisoires après inhalation de cérium-141. La relation directe entre la dose délivrée et la survie des animaux est moins évidente, bien que les résultats ne soient pas complets. On peut cependant constater qu'après inhalation de cérium-141, surtout sous forme de chlorure, on observe l'apparition de cancers du poumon à de plus faibles doses et que la survie des animaux cancéreux est en général supérieure à la durée de la vie moyenne du même groupe.

La comparaison des temps de survie des animaux cancéreux dans les différents groupes apparaît dans la figure 3. Il en ressort clairement que l'apparition des cancers du poumon se fait plus tard, mais à des doses plus faibles, après inhalation de cérium-141. Les cancers du poumon après inhalation de cérium-144 sont plus fréquents à doses faibles chez les rats

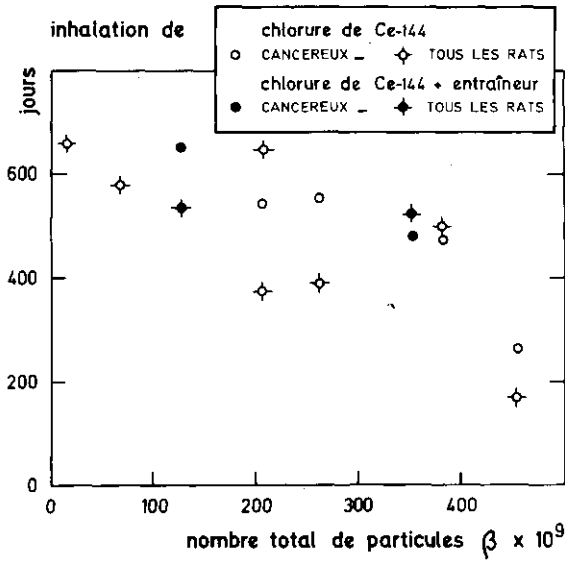


FIG.1. Relation entre l'activité cumulée dans le poumon et le temps de survie des animaux cancéreux ou non (cérium-144).

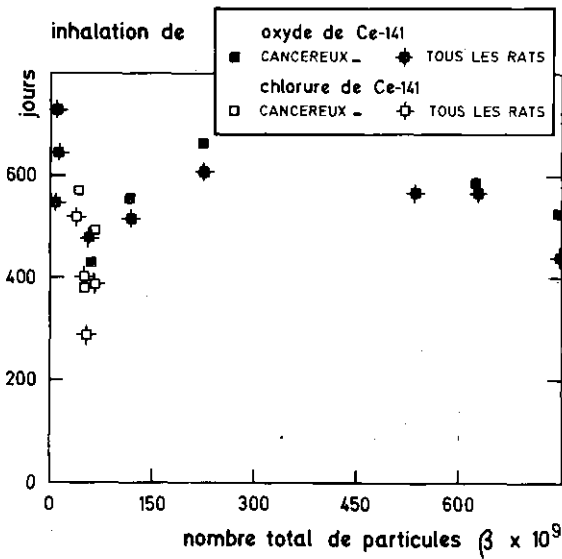


FIG.2. Relation entre l'activité cumulée dans le poumon et le temps de survie des animaux cancéreux ou non (cérium-141).

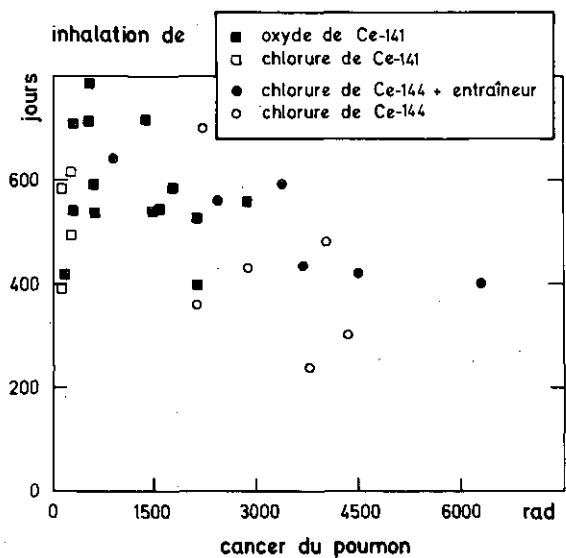


FIG.3. Relation entre la dose absorbée dans le poumon et le temps de survie des animaux cancéreux.

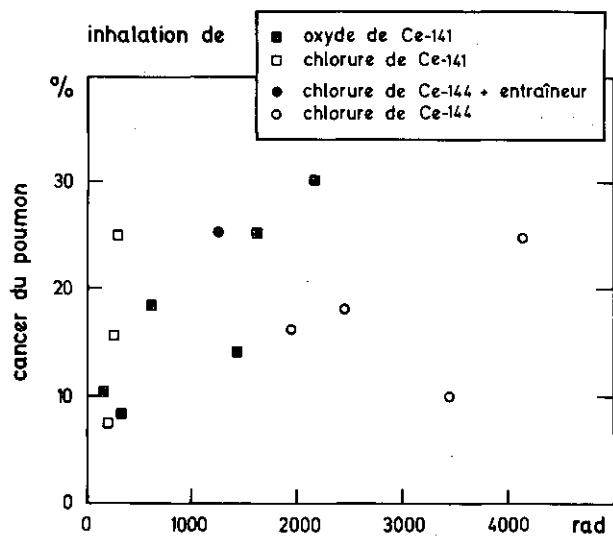


FIG.4. Fréquence des cancers du poumon en fonction de la dose absorbée.

ayant inhalé du cérium-144 avec entraîneur, ce qui est dû à une rétention beaucoup plus longue du cérium-144 avec entraîneur dans les poumons [1].

La figure 4 montre le pourcentage de cancers du poumon par groupes d'animaux en fonction de la dose administrée, exprimée en rad. Ici aussi on peut constater que l'apparition de cancers du poumon après administration de faibles doses est plus fréquente chez les animaux ayant inhalé du cérium-141 et du cérium-144 avec entraîneur.

Parallèlement nous avons entrepris des expérimentations sur l'action toxique de chlorure de cérium-144 à pH élevé après injection intramusculaire. Comme le tableau I le montre, nous avons observé chez 90 animaux ayant reçu des doses de 2000 à 10 000 nCi, ce qui correspond à des activités cumulées de 1400 à 3000 $\times 10^9$ particules émises, 56 sarcomes locaux et 11 sarcomes osseux, c'est-à-dire un pourcentage beaucoup plus grand de cancers qu'après inhalation. Ceci est dû certainement à une diffusion beaucoup plus lente du cérium après injection intramusculaire qu'après inhalation, ce qui augmente considérablement la dose locale. Une telle irradiation pulmonaire ne serait pas compatible avec une survie suffisante des animaux pour permettre l'apparition de cancers. Des doses aussi élevées diminuent la durée de vie par suite de lésions aiguës ou subaiguës de la paroi alvéolaire. Pour les doses plus faibles, les lésions spécifiques relevées après irradiation bêta peuvent se grouper en proliférations bénignes et en tumeurs malignes.

Chronologiquement les lésions bénignes prolifératives précèdent les lésions malignes. Bien qu'on ne puisse démontrer formellement que la métaplasie malpighienne ou l'adénome alvéolaire soient les précurseurs du cancer épidermoïde et du cancer bronchiolo-alvéolaire, elles semblent traduire une étape ou une impasse d'une même évolution vers l'inexorable cancérisation du tissu. Les métaplasies regroupent deux types de modification de la cellularité alvéolaire sans modification de l'architecture tissulaire que les techniques de coloration de la réticuline permettent d'extérioriser. Le premier type consiste en un recouvrement des alvéoles et canaux alvéolaires par des cellules de type bronchique cilié ou de type bronchique modifié, épidermoïde. Ce dernier type peut conduire à une accumulation intra-alvéolaire de cellules squameuses par glissement. Les masses constituées peuvent être assez volumineuses. Les mitoses sont très rares;

TABLEAU I. NOMBRE DE SARCOMES APRES INJECTION INTRAMUSCULAIRE DE CHLORURE DE CERIU-144

Nombre d'animaux	Activités cumulées (particules émises $\times 10^9$)	Sarcomes locaux	Sarcomes osseux
11	1414	7	1
50	1913	34	6
5	2486	2	0
12	2948	5	4
12	3034	8	0

TABLEAU II. NOMBRE DE CANCERS APRES INHALATION DE CERIUM-144 ET CERIUM-141

Emetteur	Nombre d'animaux	Activités cumulées (particules émises $\times 10^9$)	Carcinomes épidermoïdes	Carcinomes bronchiolo-alvéolaires	Réticulo-sarcomes	Poumons non examinés
Chlorure de cérium-144	6	20	0	0	0	0
	10	70	0	0	0	0
	18	215	1	0	1	0
	12	285	2	0	0	0
	10	385	1	0	0	0
	12	465	2	0	1	0
Chlorure de cérium-144 + entraîneur	12	135	1	1	1	2
	12	360	5	0	0	3
Chlorure de cérium-141	12	45	1	0	0	1
	12	50	0	0	0	1
	12	50	1	0	1	4
	12	65	3	0	0	2
Oxyde de cérium-141	14	10	0	0	0	0
	15	15	0	0	0	0
	10	60	1	0	0	0
	12	120	0	1	0	0
	12	230	2	0	0	6
	12	540	1	0	0	7
	12	630	1	2	0	3
	12	860	2	1	0	3

il n'y a pas de pléomorphisme. Les zones les plus fréquemment atteintes sont les alvéoles juxta-bronchiques. Le deuxième type est constitué par un revêtement continu à pneumocytes II sans fibrose interstitielle évidente.

Deux types de lésion sont proches des métaplasies: une hyperplasie générale du tissu alvéolaire orthoplasique: toutes les cellules semblent concernées à l'exception des pneumocytes I. Il existe une infiltration de la trame par quelques cellules inflammatoires: mastocytes, plasmocytes et histocytes essentiellement. Les fibroblastes et les cellules endothéliales du septum sont très augmentés. La collagénèse est discrète. Le revêtement à pneumocytes II est discontinu.

Ce type de lésion paraît spécifique de l'oxyde de cérium-141. Les lésions considérées comme provoquées par l'irradiation consistent en différentes formes de pneumonie interstitielle. Il s'agit en général du type «usual interstitial pneumonia», avec une fibrose plus ou moins sévère et un revêtement continu de l'alvéole par des pneumocytes II. Ces lésions n'intéressent que de petits territoires.

Le problème de l'adénome alvéolaire demeure un sujet de controverse. Dans notre classification il s'agit de proliférations compactes à contour nettement délimité, organisées selon une architecture propre, sans extension papillo-végétante, à cellules très orthoplasiques.

Les observations en microscopie électronique que nous avons pu faire sur des tumeurs de ce type montrent que les cellules sont des pneumocytes II identiques à ceux que l'on retrouve en majorité dans la plupart des cancers bronchiolo-alvéolaires. Il semble démontré que les proliférations identiques chez la souris sont transplantables et doivent être considérées comme malignes [5]. Ce point n'est pas acquis à ce jour en ce qui concerne le rat; c'est la raison pour laquelle nous avons conservé le groupe «adénome alvéolaire».

Les tumeurs malignes observées ne peuvent laisser aucune incertitude quant à leur malignité: si les métastases extra-thoraciques sont exceptionnelles, les invasions médiastinale et ganglionnaire sont en général de règle. Ces tumeurs ne se distinguent pas fondamentalement de celles observées après inhalation de radionucléides émetteurs alpha; cependant leur état de différenciation n'est pas analogue. Ce caractère est particulièrement net pour les cancers bronchogéniques. La plus grande partie de ces cancers est constituée de cellules indifférenciées suscitant une stroma-réaction rarement observée dans les irradiations alpha. La réaction inflammatoire et les complications septiques sont constantes. Les zones comportant une travée en cours de kératinisation ou une organisation adéno-carcinomateuse font totalement défaut. Ces caractères coïncident particulièrement bien avec les observations de Cember et Stemmer [6], tant en ce qui concerne la fréquence des cancers épidermoïdes qu'en ce qui concerne le remaniement des tumeurs et leur état indifférencié. Ce dernier caractère s'applique également aux cancers bronchiolo-alvéolaires. Les croissances papillo-végétantes sont rares dans la masse tumorale. Des formations tubulaires, constituées de cellules hautes, ressemblent quelque peu à celles de l'adéno-carcinome bronchogénique et créent aussi quelque possibilité de confusion. Ces zones sont essentiellement observées à l'extérieur du poumon, dans le médiastin où la réaction mésothéliale est pseudo-tumorale. L'invasion médiastinale précoce est un des caractères constants du carcinome bronchiolo-alvéolaire.

TABLEAU III. NOMBRE DE PROLIFERATIONS BENIGNES APRES INHALATION DE CERIU-144 ET CERIU-141

Emetteur	Nombre d'animaux	Activités cumulées (particules émises $\times 10^9$)	Métaplasies	Adénomes alvéolaires	Lésions d'irradiation	Hyperplasies	Poumons non examinés
Chlorure de cérium-144	6	20	0	0	2	0	0
	10	70	0	2	0	0	0
	18	215	0	2	1	0	0
	12	265	0	1	1	0	0
	10	385	0	0	4	0	0
	12	465	5	0	1	0	0
Chlorure de cérium 144 + entraîneur	12	135	0	0	2	0	2
	12	360	0	0	0	0	3
Chlorure de cérium-141	12	45	0	0	1	0	1
	12	50	0	1	3	0	1
	12	50	1	0	0	0	4
	12	65	0	0	0	0	2
Oxyde de cérium-141	14	10	1	0	1	2	0
	15	15	0	0	0	1	0
	10	60	0	0	0	4	0
	12	120	1	1	0	3	0
	12	230	0	0	0	0	6
	12	540	0	2	2	0	7
	12	630	0	0	1	0	3
	12	860	0	0	0	2	3

TABLEAU IV. FREQUENCE COMPAREE DES SARCOMES MUSCULAIRES ET DES TUMEURS DU POUMON
Emetteur: chlorure de cérium-144

Injection intramusculaire à pH 11			Inhalation à pH 2		
Nombre d'animaux	Activités initiales (nCi)	Nombre de sarcomes	Nombre d'animaux	Activités initiales (nCi)	Nombre de cancers
11	2960	8	18	3000	2
50	4276	40	11	4416	2
5	5654	2	10	6674	1
12	6034	9	12	7847	3
12	8558	8			

Sur le tableau II on voit le nombre de cancers du poumon par groupe après inhalation des quatre formes physico-chimiques du cérium. Sur 68 animaux ayant inhalé du chlorure de cérium-144 sans entraîneur, nous avons observé 6 cancers épidermoïdes et 2 réticulo-sarcomes. Le développement de cancers n'a été obtenu qu'à partir d'une activité cumulée de 215×10^9 particules émises. Après inhalation de chlorure de cérium-144 avec entraîneur nous avons trouvé sur 18 animaux 6 cancers épidermoïdes, 1 cancer bronchiolo-alvéolaire et 1 réticulo-sarcome. Après inhalation de chlorure de cérium-141, 5 animaux sur 40 montraient un cancer épidermoïde, et 1 sur 40 un réticulo-sarcome. Sur 80 animaux ayant inhalé l'oxyde de cérium-141 nous avons trouvé 7 cancers épidermoïdes et 4 cancers bronchiolo-alvéolaires.

Le tableau III montre les proliférations bénignes pour les mêmes groupes d'animaux. Il en sort que, à dose élevée, les métaplasies sont plus fréquentes chez les animaux ayant inhalé du cérium-144, par contre on observe beaucoup d'hyperplasies chez les animaux après inhalation de cérium-141.

Sur le tableau IV nous avons comparé la fréquence d'apparition de tumeurs malignes après injection intramusculaire et après inhalation de chlorure de cérium-144. Après administration des mêmes activités, le développement de tumeurs malignes est beaucoup plus élevé chez les animaux après injection intramusculaire qu'après inhalation.

Quand on compare les résultats que nous avons obtenus avec ceux provenant d'animaux contaminés par des émetteurs alpha on peut faire les constatations suivantes:

— Il est plus difficile d'obtenir, après inhalation, des cancers du poumon avec le cérium-144 et le cérium-141 qu'avec les émetteurs alpha. Les animaux sont plus fragiles et meurent de façon précoce à cause de l'irradiation pulmonaire générale. En effet, les animaux auxquels on administre la même activité de cérium-144 par voie intramusculaire ne présentent qu'une pathologie pulmonaire identique à celle des témoins.

- Pour un même type histologique, l'aspect microscopique des tumeurs est différent pour les émetteurs bêta et pour les émetteurs alpha.
- Le pourcentage de carcinomes épidermoïdes est plus élevé avec les émetteurs bêta.

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DISCUSSION

A. LUZ: Since you observed osteosarcomas after intramuscular injection of ^{144}Ce , I would be interested to know how large was the mean dose to the skeleton in these experimental groups.

Walburga SKUPINSKI: The bone burden was between 1 and 5 μCi . I must, however, point out that osteosarcomas were found at the place of injection. This was due, in my opinion, to the fact that cerium was injected nearer to the bone than in the case of other animals.

A. L. BROOKS: You have stated that α -emitters produce more lung tumours than ^{144}Ce or ^{141}Ce . Could you derive a RBE or effectiveness factor in producing lung tumours on the basis of rad dose to the lung?

Walburga SKUPINSKI: I think you will find the best answer to your question in the paper on α -emitters to be presented at the next session (SM-202/404).

EFFECTIVENESS OF TRITIUM AND ^{239}Pu IN PRODUCING CHROMOSOME ABERRATIONS IN Chironomus riparius*

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Abstract

EFFECTIVENESS OF TRITIUM AND ^{239}Pu IN PRODUCING CHROMOSOME ABERRATIONS IN Chironomus riparius.

The existing literature on relative biological effectiveness (RBE) indicates that the high linear energy transfer (LET) α radiation of ^{239}Pu is more effective in the production of cytogenetic effects in mammalian cells in tissue culture than are forms of low LET radiation. The purpose of this study is to compare the frequency of chromosome aberrations produced in the salivary gland chromosomes of Chironomus riparius by the low-energy β rays from tritium with those produced by the α radiation from ^{239}Pu . Adults of C. riparius were introduced into cages containing concentrations of tritiated water ranging from 30 to 250 $\mu\text{Ci/mlitre}$ and into cages containing concentrations of ^{239}Pu ranging from 0.02 to 2 $\mu\text{Ci/mlitre}$. Some larvae resulting from fertilized eggs deposited in the different containers were analysed for tritium and ^{239}Pu concentrations for dose calculation purposes while other larvae were allowed to develop into adults to produce an F_1 generation. F_1 larvae from the different concentrations of tritium and ^{239}Pu were analysed for chromosome aberrations. Aberrations were observed in larvae whose progenitors had developed in 30, 125 and 250 $\mu\text{Ci/mlitre}$ concentrations of tritiated water. The frequency produced by incorporated tritium was approximately the same as the frequency produced by an equivalent dose of chronic external γ radiation. A total of 295 F_1 larvae were analysed for chromosome aberrations from 0.02 $\mu\text{Ci/mlitre}$ concentrations of ^{239}Pu , but no aberrations were detected. The authors did not succeed in obtaining larvae for chromosomal analysis at ^{239}Pu concentration of 0.2 and 2 $\mu\text{Ci/mlitre}$; however, efforts are continuing and the possibility of plutonium metal toxicity is being investigated. The lowest calculated dose at which aberrations were detected for tritiated water was 180 rads. The dose calculated for the gonads of C. riparius in the ^{239}Pu concentration as 0.02 $\mu\text{Ci/mlitre}$ was approximately 120 rads. Based on the results obtained with tritiated water and the fact that α radiation is considered to be more effective in producing cytogenetic effects than other types of radiation, chromosome aberrations should have been detected at the dose rate calculated for the Chironomus in the plutonium culture. Thus, these preliminary data do not support a relatively high RBE for chromosome aberrations produced in the gonads of Chironomus exposed to chronic irradiation from ^{239}Pu .

INTRODUCTION

During the next several decades plutonium is expected to occupy an important position in future energy production, both as a fuel for the liquid-metal fast-breeder reactors and as a heat source in thermoelectric power systems. The toxicity of plutonium has been of concern since milligram quantities were first produced in 1943, and perhaps no single element has been so intensively studied [1]. The existing literature on the relative biological effectiveness (RBE) of high linear energy transfer (LET) radiation indicates that ^{239}Pu α emissions are more effective

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in the production of cytogenetic effects in mammalian cells in tissue culture than are low LET radiations [2]. The purpose of this study was to compare the frequency of chromosome aberrations in the salivary gland chromosomes of Chironomus riparius from the low-energy beta rays (0.018 MeV max) of incorporated tritium with the frequency of aberrations from the α emissions (5.2 MeV) of incorporated ^{239}Pu .

A comparison of the frequency of chromosome aberrations produced in C. riparius by beta radiation from incorporated tritium and chronic ^{60}Co gamma radiation was made by Blaylock [3,4]. In these studies Chironomus larvae were cultured in tritiated water and the radiation dose to larvae calculated. The frequency of chromosome aberrations was then scored in the succeeding F_1 generation cultured in non-tritiated water. In the present study a similar experiment was initiated with ^{239}Pu .

MATERIALS AND METHODS

The technique used for culturing Chironomus has been described by Biever [5] and Blaylock [3]. Plastic containers (12 cm in diameter by 13 cm in height) containing water, fine silica sand and a ground food mixture were used in the tritium and plutonium experiments. The concentrations of tritiated water used in the cultures were 30, 125 and 250 $\mu\text{Ci/ml}$. Adults of C. riparius were stocked in the culture and allowed to deposit egg masses. Water samples and larvae were collected from the different concentrations of HTO for liquid scintillation counting. These samples were counted to determine the concentration of tritium in the larvae and in the culture medium for dose calculations. The larvae which were oxidized prior to counting contained approximately the same concentration of tritium as did the surrounding water which was maintained at the specified concentration.

The beta radiation dose to the larvae was calculated by an equation from Hine and Brownell [6]. Assumptions were made that tritium was uniformly distributed in the tissue of the larvae and in the surrounding water and that the range of the β particle was small compared to the dimensions of the media in which the isotope was distributed.

The concentrations of ^{239}Pu added as a citrate were 0.02, 0.20 and 2.0 $\mu\text{Ci/ml}$ in 300 ml of water, 75 g of fine sand and approximately 15 ml of a finely ground food mixture on top of the sand. Fourth-instar larvae were collected from the 0.2 $\mu\text{Ci/ml}$ culture for plutonium analysis. Larvae were dissected to separate the gut contents and gut from the remaining body tissue. Water and sediment samples were collected at various intervals. Water samples were first centrifuged, an aliquot pipetted onto a stainless-steel planchet, and dried. Sediment was weighed, dissolved in concentrated HNO_3 with heat, then an aliquot was dried on a planchet. Body parts of Chironomus were absorbed onto filter-paper strips, which were then dissolved in concentrated HNO_3 and HClO_4 with heat. Aliquots were taken of these samples and dried on planchets. All samples were counted for α emission in a windowless gas-flow proportional counter.

The α radiation to the gonads was calculated by an equation from Hine and Brownell [6]. The calculations were made by using physical measurements of Pu concentrations in the gonads and surrounding tissue. The assumption was made that the plutonium was uniformly distributed in the gonads and surrounding tissue.

TABLE I. RADIATION-INDUCED CHROMOSOME ABERRATIONS IN Chironomus riparius

Type of Exposure	Calculated Dose (rads)	Number of Larvae	Number of Aberrations	Frequency (%)
Acute External Exposure of Adult Males to ^{60}Co	2000	257	94	36.60
Chronic Exposure of Population to ^{60}Co (~ 3 rads/hr)	1650	92	3	3.50
Larval Development in 250 $\mu\text{Ci/ml}$ HTO	1525	122	5	4.09
Larval Development in 125 $\mu\text{Ci/ml}$ HTO	760	198	3	1.51
Larval Development in 30 $\mu\text{Ci/ml}$ HTO	185	243	1	0.41
Larval Development in 0.02 $\mu\text{Ci/ml}$ ^{239}Pu	120	295	0	0

RESULTS

The concentration of ^{239}Pu in the water in each of the culture dropped to about 2% of the initial concentrations within 6 days. The food-sediment measurements indicated a concentration factor of $\sim 2 \times 10^4$. The concentration of gut content in the larvae was indistinguishable from the food-sediment concentration. The adsorption of ^{239}Pu in body tissue from food by *Chironomus* appears to result in a concentration of 0.1 to 1% of that in the diet. The variance in concentration which results following gut removal is apparently a function of the level of surface contamination on the body cuticle. Based on physical measurements of the larval organs and concentrations therein, the estimated dose rate to *Chironomus* gonads in the 0.2 $\mu\text{Ci/ml}$ culture was a maximum of 60 rads/day. The dose rates estimated for the intestinal wall and external cuticle from immersion in the food-sediment mixture in the 0.2 $\mu\text{Ci/cm}^3$ culture was 300-500 rad/day. Despite the proximity of the gonads to the intestinal wall and the small dimensions of the larvae (10-12 mm), the major contribution was from activity incorporated into the gonads, since the thickness of the intestinal wall was greater than the 38 μm range of ^{239}Pu α particles.

Table I shows the frequency of chromosome aberrations produced by the different types of radiation. The type of radiation exposure is given in column one; column two lists the total calculated dose received by the larvae. The remaining columns list the number of larvae analysed, the number of different aberrations scored and the frequency of aberrations,

respectively. Thus, the larvae that developed in 250 $\mu\text{Ci/ml}$ of HTO and accumulated a total dose of 1525 rads were progenitors of the 122 larvae in which five chromosome aberrations were detected.

The acute exposure of adult males to 2000 rads of ^{60}Co gamma radiation and the resulting high frequency 36.6% observed in the F_1 generation demonstrate that chromosome aberrations are readily introduced into the chromosomes of C. riparius (Table I). The frequency of chromosome aberrations, 3.5%, produced by exposing a population to chronic gamma radiation of ~ 3 rads/hr is close to the frequency produced in larvae developing in 250 $\mu\text{Ci/ml}$ of HTO. The lowest concentration of tritiated water at which chromosome aberrations were detected was 30 $\mu\text{Ci/ml}$. One aberration was detected in 243 larvae. In comparison, no chromosomes were detected in 295 larvae from the 0.02 $\mu\text{Ci/ml}$ culture of ^{239}Pu . Although the experiments were repeated several times, F_1 larvae could not be obtained from the 0.2 and 2.0 $\mu\text{Ci/ml}$ cultures of ^{239}Pu . At concentrations of 2.0 $\mu\text{Ci/ml}$ the fertilized eggs did not develop past the first larval instar stage. In the lower concentration of 0.2 $\mu\text{Ci/ml}$ of ^{239}Pu some larval development occurred; a few adults were obtained, but the number was not sufficient to obtain an F_1 generation.

DISCUSSION

The high frequency of chromosome aberrations produced by the exposure to acute ^{60}Co gamma radiation shows that aberrations can be readily induced into the gametes of C. riparius and transmitted to the F_1 generation. In comparison with the frequency of radiation-induced chromosome aberrations in Drosophila, which may vary from 8.7% to 16.8% for 2000 R of x ray [7,8], the frequency of aberrations (36.6%) observed for Chironomus was relatively high. Only a few studies have been conducted on induced chromosome aberrations in Chironomus; however, it appears to be a good biological system for examining the cytogenetic effects of incorporated environmental pollutants in aquatic systems.

The frequency of aberrations produced by beta radiation from 250 $\mu\text{Ci/ml}$ of HTO was very close to the frequency of aberrations produced by the exposure to chronic ^{60}Co radiation (Table I). After a review of the literature [9], Bond and Feinendegen concluded that the biological effectiveness (RBE) of incorporated tritium and ^{60}Co gamma radiation was about unity. This conclusion is supported by the data on C. riparius.

In attempting to compare the frequency of chromosome aberrations produced by α -emissions from incorporated ^{239}Pu and beta radiation from incorporated tritium, F_1 larvae from only one culture were scored. The total calculated dose received by the gonads of the larvae was 120 rads, which was less than the total dose calculated for larvae developing in the lowest concentration of tritiated water. Although no aberrations were detected in 295 larvae from the plutonium culture, a tentative statement can be made that there was no evidence to support a relatively high RBE [2,10] for chromosome aberrations produced in the gonads of Chironomus larvae exposed to chronic irradiation from incorporated ^{239}Pu . Although the radiation dose was less for the Chironomus from the ^{239}Pu culture, if α radiation is more effective in producing cytogenetic effects than other types of radiation, chromosome aberrations should have been detected in the 295 larvae from the plutonium culture. Thus, a

tentative statement can be made that in these studies there was no evidence to support a relatively high RBE [2,10] for chromosome aberrations produced in the gonads of *Chironomus* larvae exposed to chronic irradiation from incorporated ^{239}Pu . This statement must be tempered by the fact that it was not demonstrated that ^{239}Pu was incorporated into the active sites of the gonads. However, the physical measurements of the concentration of ^{239}Pu in the gonads was near that of the surrounding body tissue. Additional experiments would be necessary to determine the location of ^{239}Pu in the gonads.

Of considerable interest are the two cultures of *Chironomus* that were started at higher concentrations of ^{239}Pu (0.2 and 2.0 $\mu\text{Ci/ml}$). Although the culture at the highest concentration was stocked repeatedly with adults, and egg masses were observed in the media, larval development did not take place. It is possible that, at the initial concentration of 32 ppm of plutonium, the chemical toxicity for *Chironomus* was being reached. In any case, concentrations of 32 ppm of other heavy metals would be suspect in producing chemical toxicity to aquatic organisms. To eliminate the possibility of chemical toxicity, and to obtain data from other radiation exposure levels, additional experiments have been initiated, in which ^{238}Pu with a much higher specific activity than ^{239}Pu has been substituted for ^{239}Pu .

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DISCUSSION

A.L. BROOKS: We have done similar experiments using the liver cells in the mouse or Chinese hamster. In the mouse, after ingestion of tritium, we found an effectiveness factor of 1, while in the Chinese hamster we observed 15-20 times as many aberrations per unit dose after ^{239}Pu injection.

H. GLUBRECHT: If tritium is exchanged easily after incorporation, I wonder if you have investigated whether the effects observed after HTO uptake are due to tritium which was incorporated into other organic compounds in the cells or whether it came directly from tritium HTO.

B.G. BLAYLOCK: Since the larvae developed from fertilized eggs to adults in the tritiated water, tritium should be incorporated into the cells and the chromosomes. Autoradiography showed that tritium was indeed distributed in both. The chromosome breaks were assumed to be the result of the β radiation from the incorporated tritium. However, if tritium is incorporated into DNA, then transmutation to ^3He could produce an effect. This transmutation effect would be very difficult to measure.

M.N. KHALANSKI: The location of tritium and plutonium in chironomids could be found by using autoradiography; did you try histoautoradiography in your experiment?

B.G. BLAYLOCK: Autoradiographs were made of the larvae developing in HTO, and they showed that the tritium was equally distributed in the tissues, cells and chromosomes of the organisms. Autoradiographs were not made of the larvae developing in ^{239}Pu ; such studies are certainly needed.

GENETIC AND HAEMATOPOIETIC EFFECTS OF LONG-TERM TRITIATED WATER INGESTION IN MICE*

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Abstract

GENETIC AND HAEMATOPOIETIC EFFECTS OF LONG-TERM TRITIATED WATER INGESTION IN MICE.

With increased use of nuclear reactors for power generation, it becomes increasingly important to evaluate the possible health hazards associated with their operation. Of prime interest is the possible effect of introducing large amounts of tritiated water (HTO) from reactors into the environment. To examine this problem, randomly bred mice of the Hale-Stoner-Brookhaven strain have been maintained on HTO (3 $\mu\text{Ci/mlitre}$) for extended periods. First-generation animals on HTO from weaning (four weeks of age) have been evaluated for changes in growth pattern. Second-generation animals on HTO have been evaluated for breeding efficiency, dominant lethal mutation rate and bone-marrow integrity. A total of 18831 embryos were examined. Statistical analysis of these results using either Student's "t" test or Kruskal and Wallis' rank test indicates that there was a significant ($p < 0.01$) reduction in viable embryos and an increase in early deaths in matings involving animals drinking HTO. Beginning at eight weeks of age and monthly thereafter, the haematopoietic stem cell content of the bone marrow was determined using the exogenous spleen colony technique. Results indicate that although the total cellularity of the bone marrow remains comparable in the control and treated groups, the total number of stem cells (CFU) was reduced beginning after approximately 12-20 weeks on the tritium regime. This general reduction in CFU content continued with some fluctuation throughout the lifetime of the animal. These findings indicate a reduction in the total number of pluripotent stem cells in the marrow together with the ability of this reduced number of cells to maintain normal levels of total cellularity in the bone marrow. The results of these studies indicate that continuous ingestion of HTO at a concentration of 3 $\mu\text{Ci/mlitre}$ by mice results in: (1) Reduction in number of viable embryos present in the female at late pregnancy from matings when either the female or both parents have been on HTO; (2) Increase in number of early post-implantation deaths when both parents are on HTO; (3) Reduction in bone-marrow stem cell content after 12 weeks or longer on HTO; (4) No apparent effect on breeding efficiency (percentage of females pregnant) or body weight. These results are discussed in relation to the accumulated radiation dose.

The present and projected needs for additional electrical power in established and developing nations make it apparent that the world will of necessity become more dependent upon nuclear power reactors.

With the acceptance of nuclear power as a fact of life comes concern over the possible long-term deleterious effects arising from the radioactive by-products generated in these reactors. Of concern are the possible effects from tritium in the form of tritiated water (HTO), a major by-product of both fission and fusion reactions. A programme has been instituted in this laboratory to investigate the possible genetic (dominant lethal mutations) and somatic (haematopoietic stem cell alterations) effects in mice maintained on HTO.

* Research supported by the US Energy Research and Development Administration.

1. MATERIALS AND METHODS

All animals were randomly bred albino mice of the Hale-Stoner-Brookhaven strain. The HTO test animals were first-litter mice resulting from breedings of 8-week-old animals that had been maintained on HTO ($3 \mu\text{Ci/mlitre}$) since weaning at four weeks of age. The control animals were first-litter mice taken from the mouse colony and maintained on tap water. From the second-generation animals four experimental groups were established for dominant lethal testing [1]. Group 1 consisted of animals where both the male and female were on HTO. Group 2 consisted of females on HTO and males on tap water. Group 3 consisted of males on HTO and females on tap water. Group 4 consisted of males and females on tap water (controls). When these animals reached eight weeks of age, breeding was established by placing one male with five females for a five-day period. Fifteen days after the midpoint of this breeding period, the females were killed and the ovaries and uterine contents examined. The corpora lutea (CL) on each ovary were counted and the uterine contents classified as to viable embryos (VIA), early embryonic deaths (ED), and late embryonic deaths (LD). The early and late deaths refer to death of the embryo after implantation. EDs occurring between approximately four and ten days of pregnancy were evidenced by a small black body sometimes referred to as a "mole". The LDs occurring between approximately the tenth day and sacrifice were evidenced by a formed but dead embryo. The data from each female was placed on a computer card for subsequent analysis. New breeding groups were started each week, so a continuing programme of data accumulation took place in all four experimental groups.

The somatic effects evaluation was done by examining the size and competence of the haematopoietic stem-cell pool from second-generation animals maintained on either HTO or tap water for 80 weeks. At approximately four-week intervals, animals were sacrificed and the bone marrow removed from the hind legs (femora and tibia) using the quantitative technique of Stoner and Bond [2]. Determinations were made on at least four animals at each point, and the average total nucleated cell content was determined. The number of stem cells/leg was then determined using the spleen-colony technique of Till and McCulloch [3]. This method involves injecting a known number of bone-marrow cells into a recipient mouse that has received a single whole-body 250-kVp X-ray exposure of 750 rads within 24 hours previous to the bone marrow injection. After 7 days the recipient animals were killed, their spleens removed, fixed in Bouin's solution, and the number of surface colonies determined. The colony number is directly related to the number of pluripotent stem cells in the injected sample. In addition to the genetic and haematopoietic determinations, weight records were kept on previously selected groups of mice to determine any effect on body growth.

2. DOSIMETRY

At selected times after being placed on the tritium regimen, animals were sacrificed and the amount of tritium in the blood plasma and various soft tissues determined by scintillation counting. Calculations of accumulated dose were based on these determinations.

TABLE I. DOMINANT LETHAL ANALYSIS
PARAMETERS

Mean Values on Tritium Animals 3 μ Ci/ml HTO								
	Control (C)		Male-Female		Female only		Male only	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Cl	10.967	0.071	10.564	0.052	10.666	0.084	10.718	0.079
Via	8.986	0.105	8.146	0.075	8.257	0.121	8.402	0.118
ED	0.470	0.036	0.623	0.033	0.546	0.046	0.563	0.044
LD	0.038	0.011	0.050	0.008	0.038	0.012	0.057	0.014
Pre	1.473	0.096	1.748	0.068	1.825	0.108	1.696	0.108
No. Mice	Bred	690		1531		612		621
	Preg.	366		764		315		316
% Preg.	53.04		49.90		51.47		50.89	

3. RESULTS

3.1. Genetic effects

A total of more than 3450 animals were bred in the four treatment groups. There was no significant difference in the percentage of pregnant females in any of the four groups. The mean values for the number of viable embryos, early deaths, late deaths, and pre-implantation deaths are given in Table I.

The significance of the differences between various experimental groups was then tested using three statistical tests:

- (1) Student's "t" test [4], a parametric test which assumes normal distribution of the data. In our analysis this test makes use of the pooled data of all groups.
- (2) The rank test of Kruskal and Wallis [5], a non-parametric test for a complete random design with any number of populations. The final analysis in this test was made using a χ^2 test.
- (3) The arcsine transformation of Salsberg [6]. This test normalizes the data and computes the mutation index for each treatment, which may then be compared using a χ^2 test.

The results of all three analyses were consistent, indicating a significant difference in the number of viable embryos between the control group when compared with either group 1 (male and female on HTO) or group 2 (female only on HTO). Similarly, a significant difference was seen in the number of early deaths when comparing the control group with group 1.

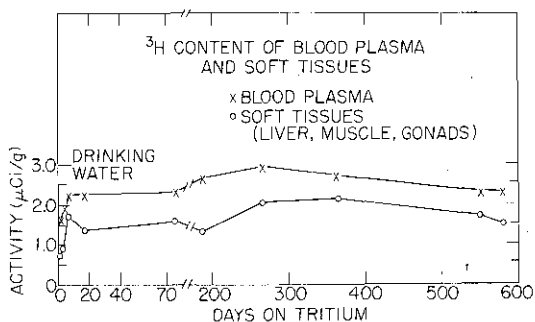


FIG. 1. Tritium incorporation into blood plasma and soft tissues.

3.2. Stem cell determinations

No significant difference in total cellularity of the leg bone marrow was noted between the HTO and control animals. However, when the relative number of pluripotent stem cells (CFU) and the total number of CFU/leg were determined, marked differences were noted. The first depression in total CFU/leg was evident as early as twelve weeks of age in the second-generation animals on tritium. With some variation this depression continued throughout the remainder of the 80-week observation period.

3.3. Dosimetry

After the initial increase in tritium concentration in blood plasma and soft tissue, a relative equilibrium was maintained throughout an observation period of 570 days (Fig.1). Dosimetry calculations were made on the basis of the average tritium content over the period from 17 to 265 days. These values in $\mu\text{Ci/g}$ were: blood plasma = 2.36; soft tissues (liver, spleen and gonads) = 1.61. If these values are converted to rad/d absorbed dose, average soft tissues = 0.47 rad/d. The activity in other tissues varied directly with the water content of the tissue. In ovaries the rad dose per day was 0.36 and in testes 0.48.

Using the blood plasma value for calculating the dose to the bone marrow, the accumulated dose for the first 25 weeks (onset of significant reduction in CFU content) was approximately 120 rads. If a similar calculation is made for the second-generation animals' ovaries and the resulting embryos, the value is 31.3 rads. This calculation assumes: the first appearance of the ovaries at the eighth day of gestation in the first-generation animal; 56 days of aging after the birth of the second-generation female; a five-day breeding period and 15 days until sacrifice. This is a most conservative estimate (overestimation of dose), since the radiation dose delivered during the final days in the pregnancy of the second-generation animals could not contribute to the early death findings. Measurements of body weight indicated no difference between HTO and control animals.

4. DISCUSSION

The possible genetic and somatic effects of chronic ingestion of HTO (3 $\mu\text{Ci/mlitre}$) have been investigated in mice. A significant reduction in

the number of viable embryos resulting from matings of animals with both partners on tritium and in matings where only the female was on tritium have been observed. Similarly, an increase in the number of early deaths was noted in matings where both partners were on tritium. Since the reduction in viable embryos is due to a number of factors (early death, late death and pre-implantation loss), it is not surprising that an effect on the number of viables would be seen in both groups 1 and 2; whereas, for the early deaths, an effect was seen only when both mating partners were on tritium. Continuing replacement of mature sperm in the male limits the total accumulated exposure to these cells, whereas in the female the dose accumulation persisted in the second-generation animals beginning in utero and continuing throughout pregnancy.

The positive effects seen using the somewhat insensitive dominant lethal test system and the effects seen on the blood-forming cells indicate that, at least in the mouse, there is a hazard in the continuous ingestion of HTO at a concentration of $3 \mu\text{Ci/mlitre}$. A direct comparison of these results with a human drinking an equivalent amount of HTO is impossible due to the obvious differences in water metabolism between the two species. Until further experimentation at lower levels of ingestion are completed it is difficult to comment on the significance of these results as related to current concepts of maximum permissible concentration. Studies are now under way examining the possible effects of lower concentrations of chronic HTO ingestion.

ACKNOWLEDGEMENTS

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DISCUSSION

K.L. MOSSMAN: Have you included microscopic colony counts in your study of haemopoietic effects?

A.L. CARSTEN: No, we have not.

K.G. LÜNING: Referring to your analysis of dominant lethals, I must say that you have been too modest about the conclusions. When you use the percentage of intra-uterine deaths as the basis for comparison, it is

obvious that your material in groups 2 and 3 indicates effects of HTO of about the same magnitude in both sexes. The effects observed in group 1 are nearly twice those in group 2 or 3. So your data clearly demonstrate the effects of HTO in both sexes. I hope you will continue this important investigation.

A.L. CARSTEN: Thank you. Your evaluation indicates an even greater effect than we have suggested in our conservative analysis and emphasizes the need to examine lower concentrations. Studies on the effects at $1.0 \mu\text{Ci/ml}$ are now under way, and the results will certainly be considered in the light of your comments.

H.H. VOGEL, Jr.: Did you find any significant reduction in living young mice between birth and weaning after ^3H exposure? I ask this question because in Sprague-Dawley rats subjected to a single whole-body exposure of 50-rad fission neutrons in utero on the 18th day of gestation, some 25-30% die between birth and weaning.

A.L. CARSTEN: This analysis is still preliminary, but at the moment we see no effect.

E.J. AINSWORTH: Have you observed any correlation between a reduced femur CFU content and levels of circulating platelets? We observe significant reductions in platelet counts in age-irradiated animals given single or fractionated doses of fission neutron or γ radiation; the CFU content in the femur is also significantly reduced in these circumstances.

A.L. CARSTEN: We have not looked at the levels of circulating platelets. This may well be a more sensitive factor, which we could examine in our current study where animals are being maintained at lower levels of tritiated water.

J.M. HOLLAND: Since estimations of pre-implantation loss using corpora lutea (CL) counts are subject to error not only in discerning individual CL and distinguishing CL of pregnancy from CL of oestrus but also in assessing the importance of oligospermia, have you taken the last-named factor (oligospermia) into consideration in your calculations?

A.L. CARSTEN: Although we are aware of the problems of estimating pre-implantation loss, we have made no allowance for this since there appears to be no effect involved. Dr. Lüning's analysis, which ignores CL counts and pre-implantation loss, indicates a positive effect, which agrees with our analysis.

J.M. HOLLAND: Would you please comment on the apparent paradox whereby your dominant lethal data show an effect (post-implantation) when both sexes were treated but not when each sex was treated independently?

A.L. CARSTEN: Our data indicate an effect at $p < 0.01$ when the females are treated and at $p < 0.05$ when either sex is treated. As Dr. Lüning has pointed out, in his method of analysis there is an effect for males and for females treated, and these effects are added in order to obtain the result for both groups.

M. GOLDMAN (Co-chairman): Do you attribute any significance to the apparent cycling of the CFU yield at late times in the study? Did the colonies (size and type) reflect a comparable periodicity?

A.L. CARSTEN: No, we do not attach any significance to this phenomenon. We have not made a detailed study of size and type of colonies. Our impression - albeit only subjective - is that there is no difference in colony size.

LOW-LEVEL CHRONIC EXPOSURE TO TRITIUM

An improved basis for hazard evaluation*

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Abstract

LOW-LEVEL CHRONIC EXPOSURE TO TRITIUM: AN IMPROVED BASIS FOR HAZARD EVALUATION.

The possibility that significant biological effects may result from chronic low-level exposure to $^3\text{H}\text{OH}$ is the main health and environmental concern regarding tritium from nuclear energy operations. Direct data for such exposure are, however, largely still unavailable. Information on chronic irradiation at low levels derives mostly from γ -ray studies, so extrapolations are necessary for tritium. But controversy exists on tritium's relative biological effectiveness (RBE) compared to γ radiation and on the related quality factor (Q) used for radiation protection (recently adjusted downward from 1.7 to 1). A firmer basis for extrapolation is needed. The author has obtained quantitative data for low-level $^3\text{H}\text{OH}$ and ^{60}Co γ -ray exposures in the intact mammal. Developing mice were exposed to various doses over 33 days; surviving oocytes were then counted in ovaries and compared with controls, providing dose-response curves for both radiations. By comparing these curves, RBE values were determined. The RBE was found to vary inversely with dose, in accord with the theory of dual radiation action. At γ -ray doses of 50 rads, the RBE was 1.6. It increased at lower doses, reaching a value of approximately 3. With short exposures, the RBE was lower, due possibly to differences in microdistribution of ^3H . These results demonstrate that conclusions from both high-dose and short-term experiments are likely to underestimate the effects of low-level chronic exposure to $^3\text{H}\text{OH}$. Measurement of the systematic variation of tritium's RBE with dose and recognition of the effect of exposure duration provide a more secure basis for hazard evaluation.

1. INTRODUCTION

Tritium has low radiotoxicity compared to most other radioactive nuclides [1], especially compared to such very dangerous material as plutonium. However, large quantities of ^3H are involved in nuclear energy operations [2, 3]. It is a prominent by-product of nuclear fission, difficult and costly to contain, and impressive amounts find their way to the environment [4]. Further, tritium is an important fuel for nuclear fusion, and if the fusion reaction becomes successfully harnessed for peaceful purposes, ^3H will assume an even more significant role in the world's technology than it has today. Tritium is the radioactive isotope of the living cell's most abundant, most ubiquitous atom, and in its preferred state, water, it has unimpeded access to living things. Decreased relative brain weight in rats [5] and decreased numbers of female germ cells in mice [6] have been reported following chronic exposure to $^3\text{H}\text{OH}$ at levels in body water as low as $0.1 \mu\text{Ci/ml}$. These considerations make it imperative that the possibility of deleterious effects from low levels of $^3\text{H}\text{OH}$ on human health or on the environment be thoroughly evaluated.

* Work performed under the auspices of US Energy Research and Development Administration.

It was for these various reasons that we undertook a fresh reexamination of tritium toxicity, with emphasis on low-level exposure. Since much work had been done in the past on the behavior of tritium in the body and its effects on the adult, we focussed attention on quantitative in-vivo cell-population studies in the developing animal. Results from studies on primary oocytes in the ovaries of prepubertal mice have given insight into the radiobiology of low-level ^3HOH exposure, especially the tritium RBE. Highlights of these results are summarized here.

2. EFFECTS OF ^3HOH ON MOUSE OOCYTES

Primary oocytes in sexually immature mice and rats are cells of extraordinary radiosensitivity. In the mouse, their LD_{50} for acute gamma irradiation is less than 10 rads [7]. Cahill and Yuile [8] observed decreases in ovarian size in newborn rats exposed in utero to ^3HOH at body-water levels of $20 \mu\text{Ci/ml}$. Other investigators have reported marked reductions in oocyte numbers in rats following administration of ^3H -thymidine [9, 10] and of ^3HOH [11, 12] during fetal development.

In order to obtain more exact, quantitative dose-response data at low exposure, we administered ^3HOH in drinking water to mother mice throughout pregnancy and lactation. Body-water levels were measured by radioassay of urine samples, and oocyte numbers in the continuously exposed offspring were determined at 14 days after birth by microscopic counting in serial sections of ovaries, and compared to controls. Details of experimental methods and results have been described elsewhere [6, 13, 14, 15]. Fig. 1 shows the relation found between oocyte survival and ^3HOH concentration in body water.

These combined results from several studies demonstrate that: oocyte survival decreases exponentially with dose; there is no threshold; and the LD_{50} level is about $2 \mu\text{Ci/ml}$ body water.

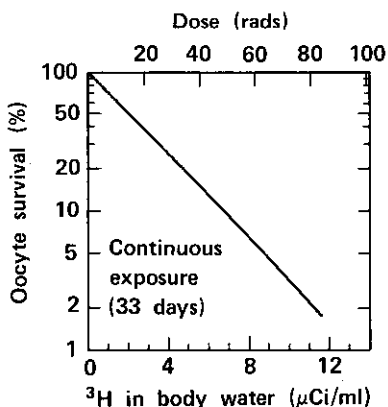


FIG.1. Oocyte response to ^3HOH exposure. Survival of primary oocytes shown as a function of tritium concentration in body water and of the corresponding dose in rads.

3. EFFECTS OF ^{60}Co GAMMA RAYS ON MOUSE OOCYTES

Since such striking biological effects — manifest as cell killing in the intact mammal — were found at such low levels of ^3HOH , it was important to ascertain whether oocyte responses to a more "standard" radiation, such

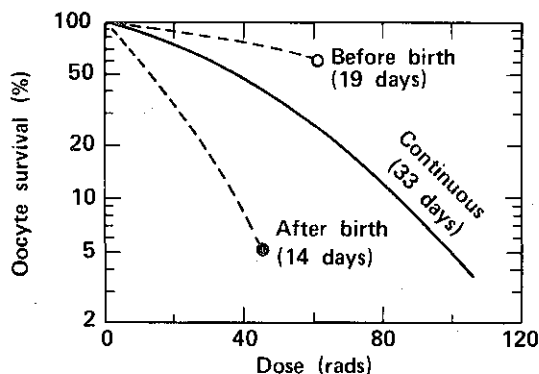


FIG.2. Oocyte response to ^{60}Co γ -ray exposure. Survival of primary oocytes shown as a function of dose. Solid line: continuous exposure from conception to 14 days after birth.

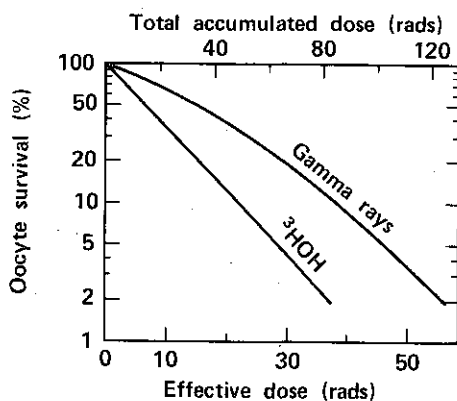


FIG.3. Oocyte responses to ^3HOH and ^{60}Co γ -ray exposure compared.

as gamma rays, would be significantly different. Comparative studies, described in detail elsewhere [13, 14, 15], were therefore carried out using ^{60}Co gamma rays. Mice were exposed continuously from conception to 14 days after birth, as in the case of ^3HOH , and surviving oocytes were enumerated in ovaries and compared to controls.

A typical dose-response curve is shown in Fig. 2 (solid line). In contrast to that for ^3HOH (see Fig. 1), the gamma-ray curve is not exponential. It is upwardly convex, indicating that the effectiveness of gamma radiation decreases at low doses. This is in agreement with the theory of dual radiation action [16].

In Fig. 2 it may be seen also that irradiation from birth to 14 days of age results in much greater oocyte killing than exposure confined to the 19-day period from conception to birth. Most of the effect described by the solid line in Fig. 2 is therefore due actually to a smaller dose than indicated on the scale. The "effective" dose, under conditions of continuous exposure from conception, is 45% of the total accumulated 33-day dose.

4. ^3HOH AND GAMMA RAYS COMPARED — THE RBE

When all our data on continuous, 33-day exposures for ^3HOH and ^{60}Co gamma rays are pooled, as appropriate, and considered together [15], the two curves shown in Fig. 3 are obtained. The ratio of doses from gamma rays and ^3HOH that produce the same effect is the RBE. From Fig. 3 it is seen that the RBE is not constant. It varies inversely with dose. An effective gamma-ray dose of 50 rads reduces oocyte survival to 3.5%; the same 3.5% survival results from an effective ^3HOH dose of 31 rads: an RBE of 1.6. At 20 rads the RBE is 2. It continues to rise at lower doses, and according to the curves in Fig. 3 reaches a value of 2.8.

From comparisons between individual gamma-ray and ^3HOH experiments we obtain limiting RBE values ranging from 2.5 to 4.2. While there is some uncertainty concerning the exact value reached as doses approach zero, it may be said to be approximately 3.

5. PROTRACTED AND SHORT EXPOSURES COMPARED

Comparative studies have also been carried out using shorter (5-day) exposures to both ^3HOH and ^{60}Co gamma rays. These have been described in detail elsewhere [17]. Irradiations by tritium and gamma rays at 4.1 and 5.9 rads/day respectively were started when mice were 20 days old (at the time of weaning) and continued until they were 25 days old. At that time oocyte survival was determined. The results are shown in Fig. 4, where they may be compared with those from chronic exposure. Higher oocyte survival seen with the 5-day exposures — for both ^3HOH and gamma rays — is due to decreased oocyte radiosensitivity in the older mice. This does not affect the RBE. As seen in Fig. 4, the RBE for short exposures is 1.4 at 30 rads of gamma rays. For protracted exposure, at the same gamma-ray dose, it is 1.9.

While it is possible that this difference in RBE may be due to the differing dose rates used for the short and long exposures, contrary arguments may be marshalled [17]. Alternatively, the greater RBE found with chronic exposure may be due to differences in microdistribution of tritium atoms within the cell. Protracted exposure, particularly during periods of rapid growth and development, provides more opportunity for metabolic turnover and incorporation of tritium into diverse molecules.

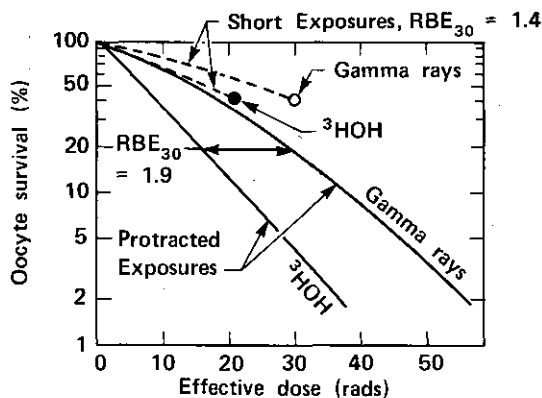


FIG.4. Oocyte responses to ^3HOH and ^{60}Co γ rays given in short (5-day) and protracted (33-day) exposures compared.

The results seen here suggest that tritium given as ^3HOH may, with time, become located in radiobiologically more sensitive sites. Regardless of the mechanisms involved, however, this finding shows that tritium is relatively more effective when exposure is protracted.

6. DISCUSSION AND CONCLUSIONS

While it is recognized that observed values of RBE may depend upon the experimental conditions used and the biological end-points measured, there has nevertheless been a disturbing lack of agreement, and some confusion, concerning tritium's RBE compared to gamma rays, and concerning the related Quality Factor (Q), used for radiation protection [18]. Various RBE values for tritium have been reported. Those determined in mammalian systems have for the most part ranged from 1 to somewhat more than 2, although higher values have been suggested by certain data [19], and challenged by others [20]. The value assigned to Q has recently been adjusted downward from 1.7 to 1 [21]. It has been well argued, however, that a value of 2 might be more appropriate [18].

The results reported here demonstrate that dose has an important influence on RBE, especially in the low-dose range. This is in accord with theory [16] and with microdosimetric measurements on ^3H beta particles and ^{60}Co gamma rays [22]. The results summarized here are from studies on low-level exposure, using a very sensitive in-vivo mammalian system. They show that differing values of RBE can be expected from experiments carried out at different regions of the dose range. Most important from the practical point of view of radiation protection is the fact that at low-level exposures, delivering small effective doses, the RBE of tritium is elevated. While it may approach unity at large doses, such as are frequently used in radiobiological studies, it is more nearly 3 at low doses which are of more practical concern from the radiation protection standpoint. Also, radiobiological experiments employing short-term exposures may yield spuriously low RBE values, and thereby underestimate tritium hazards. It is comforting, on the other hand, to note that the present results give no indication that the tritium RBE at low exposure levels appreciably exceeds 4.

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DISCUSSION

C.E. EASTERLY: Have you attempted to assay the oocytes for tritium concentration?

R.L. DOBSON: We routinely measure tritium concentration in body water. We have, however, also made determinations of tritium concentrations in soft tissue, including ovary, differentiating between tritium in tissue water and tritium in non-water components. We have not attempted measurement of tritium levels in isolated oocytes, i.e. in oocytes separated from ovarian tissue.

Y. NISHIWAKI: What do you think of the relative significance of the transmutation effects of tritium, i.e. the effects due to disintegration of tritium in important biological materials such as the nuclear material of the cell, especially after administration of ^3H -thymidine?

R.L. DOBSON: This is an interesting point. However, I hesitate to comment on it since the data I have presented here do not bear upon this question. We administered tritiated water to our experimental animals, and our results do not provide information on transmutation effect.

D.G. WILLHOIT: What was the dose rate of the protracted ^{60}Co exposures?

R.L. DOBSON: It varied from slightly less than 1 rad/d to somewhat more than 3.5 rad/d.

B.G. BLAYLOCK: You mentioned some uncertainties about tritium. Would you please comment on whether tritium incorporated in food and eaten by the organism would occupy sites in the organism which would not be occupied by tritium taken in as HTO?

R.L. DOBSON: The cell is mostly water (about 70%), and the radiation dose, in exposures such as those we used, will be primarily from tritium in cell water. With protracted exposures to ingested ^3HOH , however, about 8% of the dose will come from tritium incorporated in non-water cell components. If tritiated food is ingested as well, this dose component may be expected to be larger (but not more than 20% of the total), and it is possible that tritium will have a slightly different distribution in organic molecules. In our studies, the primary exposure was to tritium in drinking water.

M. GOLDMAN (Co-chairman): Could you please clarify how the dose to oocytes from ^3HOH was measured?

R.L. DOBSON: We compute the dose in two parts — the dose delivered by tritium in cell water and that from tritium which is incorporated in non-water cell components. Data from various sources (including our own studies) indicate that the cells of the soft tissues of mice consist of nearly 70% water. There will, of course, be variations. We take cells (the oocytes) to be 70% water. They will therefore receive (from their own cell water) 70% of the dose which "pure" body water would give to itself. The non-water portion of the cell (30%) is taken as being tritiated to a level of 30% of that of body water. This is based on data from various sources, including the studies of Cahill and co-workers, ourselves and others. Combining the two dose contributions (0.204 and 0.017 rad/d respectively) and assuming uniform distribution of ^3H , we find that radiation will be delivered at 0.22 rad/d if the body-water tritium level is $1\text{ }\mu\text{Ci/mlitre}$.

BIOLOGICAL ASSESSMENT OF CONTINUOUS EXPOSURE TO TRITIUM AND LEAD IN THE RAT

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Abstract

BIOLOGICAL ASSESSMENT OF CONTINUOUS EXPOSURE TO TRITIUM AND LEAD IN THE RAT.

A broad investigation of the effects of simultaneous exposure to two potentially synergistic environmental pollutants, tritiated water (HTO) and lead, was conducted. Sprague-Dawley rats were continuously exposed to HTO and/or Pb in drinking water from conception of the F_1 through adulthood of the F_2 generation. A 12-cell exposure matrix was used employing HTO activities calculated to provide approximately 3-300 mrad/d whole-body irradiation and Pb levels of 5 or 50 ppm in drinking water. Observations were made on the reproductive capacity of the F_1 generation and the effects of lifetime parental exposure to HTO and/or lead on the F_2 neonates. The effects of single and combined exposures on the development and function of the central nervous system, some brain catecholamine levels and electroencephalogram patterns were also examined in both generations. The results indicate that, in both generations, continuous HTO exposures as low as 3 mrad/d delayed development of righting reflexes in young rats; 30 mrad/d additionally depressed the spontaneous activity of adult male rats. Continuous exposure to 5 ppm lead produced similar effects on righting reflex development and adult spontaneous activity. The relative brain weight of F_2 neonates was decreased after lifetime parental exposure to 300 mrad/d or 5 and 50 ppm lead. Chronic lead exposure also appears to induce superovulation and increase preimplantation deaths in F_1 dams. Dose-effect responses to both HTO and lead were less than additive in their interactive effects on the parameters measured.

INTRODUCTION

In an attempt to contribute information on the effects of simultaneous exposure to two potentially synergistic environmental pollutants we have chronically administered tritiated water (HTO) and lead (Pb) to rats. HTO and Pb were selected because they are ubiquitous in the environment; technological advances have significantly increased their environmental levels; both are transplacentally transferred [1,2] and can affect intrauterine development [3,4]. Further, exposure to radiation or Pb in utero can produce postnatal neurological and reproductive dysfunction [3-6]. The chronic exposure levels employed were 0.01 - 1.0 μ Ci HTO/ml body water (3-300 millirads/day) and 5 and 50 ppm of Pb in drinking water. These are, respectively, 2-200 times MPC_w for an individual in the general population [7] and 100-1000 times the U.S. Drinking Water Standards [8].

MATERIALS AND METHODS

Male and female Sprague-Dawley rats (Blue Spruce Farms, Altamont, NY) ages 50-60 days, were randomly assigned to one of three treatment groups: 0, 5 or 50 ppm Pb (acetate) administered in deionized drinking water for 40 days prior to mating. When a positive sperm determination was made

these P_0 females were assigned to one of four HTO groups: 0, .01, .10 or 1.0 $\mu\text{Ci/ml}$ and injected i.p. with sufficient HTO to establish the desired body-water activity. HTO activities in body water were maintained by ad libitum access to HTO drinking-water supplies. The result was a 12-cell exposure matrix of pregnant P_0 rats exposed to HTO only, Pb only or combinations of HTO and Pb.

F_1 progeny were thus continuously exposed to the HTO and/or Pb regimens from conception through gestation and postnatal life. At 100 days, the F_1 rats were mated and some F_2 litters were delivered by Caesareotomy at full term for observation of the F_2 neonates. P_0 and F_1 dams and the F_2 female offspring were removed from the study at weaning. F_1 and F_2 male rats continued to be exposed until 180 days of age. Naturally delivered litters in both the F_1 and F_2 generations were reduced to 8 pups per dam on day 1 and weaned on day 21.

OBSERVATIONS ON F_2 NEONATES

Following delivery of the F_2 by Caesareotomy, the ovaries from 6-8 dams per dose group were removed for corpora lutea examination and the uteri examined for resorption sites. F_1 maternal blood, liver and kidneys were taken for Pb analyses. Litter size was recorded, the fetuses sexed, examined for topographical anomalies and weighed. The brain, heart, lung, liver, kidneys and spleen from two males and two females in each litter were removed, weighed and frozen for subsequent Pb analysis. Pooled blood samples were obtained from the remaining pups in each litter for Pb determinations.

OBSERVATIONS ON ADULT F_1 AND F_2 MALES

Six F_1 and F_2 males per treatment group were sacrificed at 180 days of age and body weight recorded. Blood, brain, liver, kidneys, testes and femur were removed, weighed and frozen for subsequent lead analysis. Catecholamine analyses were also performed on whole brain. Complete gross and histopathologic evaluations were performed on these males.

LEAD AND TRITIUM ANALYSIS

HTO activities in each batch of drinking water were determined by liquid scintillation counting. The average body-water HTO activity for the 2-3 rats in each cage was determined from composite 24-hour urine samples collected biweekly.

Pb determinations were done with a Perkin-Elmer model 306 atomic absorption spectrophotometer (AAS) with a three-slot burner head and deuterium background corrector. The Pb content of each batch of control and Pb water supplies was verified by AAS analysis. Blood and tissue Pb concentrations were determined by the Delves cup method of Fernandes and Kahn [9]. Bone and food samples were analysed by the method of Yeager et al. [10]. Adequacy of the analytical procedures was evaluated by assaying a National Bureau of Standards Bovine Liver (No. 1577) by the methods outlined above.

Delta aminolevulinic acid (ΔALA) was measured in urine by the method of Marver et al. [11].

DEVELOPMENTAL TESTING

Five pups from each of 4-6 litters per treatment group in both the F_1 and F_2 generations were tested for the ages at which the startle response, the righting reflex and eye opening developed. The startle response was evoked by a click (generated by a toy clicker) sounded immediately behind the head of an animal held suspended by the nape of the neck. A positive response consisted of a muscle jerk in any of the extremities.

The righting reflex was elicited by holding the rat upside down by the nape of the neck and the tail and dropping it onto wood shavings from a height of approximately 30 cm. A response was considered positive if the animal righted in the air and landed on all four feet.

Age at eye opening was recorded upon complete opening of both palpebral fissures.

LOCOMOTOR ACTIVITY

The locomotor activity of adult F_1 and F_2 males was recorded at approximately 165 days of age in a residential maze as previously described [12]. Groups of three littermates from 5 litters per dose group in both generations were tested in the maze for a period of 23 hours per day for 4 consecutive days. The mazes were housed in a sound-attenuated room maintained on a 12-hour light/dark cycle beginning at 6:00am. Food and water were available ad libitum. Activity counts were recorded utilizing a Xerox mini-computer. Data obtained on days 2-4 were averaged to obtain mean daily activity levels for litters. The average of the 5 litter averages was used to compute treatment means.

CATECHOLAMINE ASSAYS

In both the F_1 and F_2 generations, 6-8 males and females from each dose group (28 days old) were decapitated and 5-10 adult male rats (180 days old) were killed by pentobarbital injection and decapitated. Whole brains were removed, weighed and immediately frozen on dry ice. Catecholamines were isolated from whole brain on aluminum oxide following the method of Anton and Sayre [13]. Norepinephrine (NE) was assayed fluorometrically according to von Euler and Lishajko [14] and dopamine (DA) by the method of Barchas, Erdelyi and Angwin [15].

ELECTROENCEPHALOGRAMS (EEG)

EEGs of 180-day-old F_1 and F_2 male rats were measured. Rats were anesthetized with sodium pentobarbital (40 mg/kg) i.p. After loss of the blink reflex and attainment of abdominal breathing, platinum needle electrodes were placed subcutaneously in the scalp at mid-frontal (MF) and right and left occipito-parietal (RO and LO) locations. Bipolar recordings were made from the MF x RO lead-pair on a Beckman Dynograph. Simultaneously, the amplified signal was subjected to interval analysis using a DEC Lab-8 computer. Two 2-1/2 minute segments of anesthetized EEG were machine-processed to provide the zero-potential crossover (ZPC) rate per 5-second epoch, and the percent abundance of waveforms in β (13-50 Hz), α (7.7-12.5 Hz), θ (4-8 Hz) and δ (<4 Hz) frequency classes.

STATISTICAL ANALYSIS

HTO or Pb effects were analysed by a one-way analysis of variance (ANOVA) and a t-test. HTO/Pb interaction was tested by a two-way ANOVA with the null hypothesis that the interaction was additive and linear.

RESULTS

Throughout the experiment the HTO activities and Pb levels in the drinking water were maintained within $\pm 10\%$ of the design levels. Further, the Pb levels in deionized drinking water were < 0.01 ppm, in ambient air < 0.1 $\mu\text{g}/\text{M}^3$, and in rat food an average of 0.9 ppm.

HTO had no effect on the growth of the F_1 or F_2 although a transient body-weight deficit was observed at ages 9-15¹ days in the F_2 generations. In both generations, rats exposed to Pb only or HTO/Pb were not significantly different in body weight from controls at any age nor did exposures to Pb produce overt signs of Pb intoxication. Hematocrits of adult F_1 rats were indistinguishable from controls but the ΔALA level in the urine of adult F_1 rats exposed to 50 ppm was approximately 50% higher than control values.

The reproductive data on F_1 dams is presented in Table I. No effects were seen on the parameters listed as a result of HTO exposure or the HTO/Pb interaction. The results do indicate, however, that chronic Pb exposure to 5 ppm increased litter size significantly while exposure to 5 or 50 ppm Pb significantly increased the corpora lutea count and at 50 ppm preimplantation deaths were increased significantly.

Neither HTO, Pb nor the combinations affected F_2 neonatal body weight or the absolute or relative weights of the neonatal liver, kidney, heart or spleen. When pooled across HTO exposures, the 50-ppm Pb lungs weighed about 10% less than controls, although this was not statistically significant.

Table I
Reproductive Performance of F_1 Dams^a

Pb Level	Litter Size	% Resorptions	Corpora Lutea	Average Pre-implantation Deaths Per Litter	
				(all litters)	(litters with PID ^b)
0 ppm	(32) ^c 10.7 \pm 0.3 ^d	(31) 0.65 \pm 0.12	(29) 12.1 \pm 0.4	(29) 0.93 \pm 0.26	(15) 1.8 \pm 0.4
5 ppm	(37) 11.8 \pm 0.3 ^f	(37) 0.62 \pm 0.11	(32) 13.8 \pm 0.5 ^e	(32) 1.6 \pm 0.4	(16) 3.2 \pm 0.5
50 ppm	(39) 11.0 \pm 0.3	(39) 0.64 \pm 0.11	(38) 14.0 \pm 0.5 ^f	(38) 2.3 \pm 0.4 ^e	(26) 3.4 \pm 0.5 ^e

a = No tritium or lead-tritium interaction effects were detectable. Data have been pooled by lead groups.

b = PID = Corpora Lutea - (Live + Resorptions)

c = sample number

d = mean \pm SE

e = $p < 0.05$ by ANOVA and t-test

f = $p < 0.01$ by ANOVA and t-test

Table II

Relative % Brain Weight of F_2 Neonates^a

Treatment	0.0 μ Ci/ml	0.01 μ Ci/ml	0.10 μ Ci/ml	1.0 μ Ci/ml
0 ppm	(6) ^b 4.23 \pm 0.09 ^c	(6) 4.15 \pm 0.06	(6) 4.03 \pm 0.10	(7) 3.92 \pm 0.05 ^e
5 ppm	(6) 3.94 \pm 0.08 ^d	(7) 4.10 \pm 0.08	(6) 4.08 \pm 0.06	(5) 4.19 \pm 0.08
50 ppm	(6) 3.94 \pm 0.05 ^d	(6) 3.90 \pm 0.03 ^e	(6) 4.23 \pm 0.07	(6) 4.12 \pm 0.09

a = Interaction of HTO and Pb was significant by two-way ANOVA

b = number of litters

c = mean of litter means \pm SEd = $p < 0.05$ by ANOVA and t-teste = $p < 0.01$ by ANOVA and t-test

The relative brain weight of F_2 neonates is presented in matrix form in Table II. HTO produced a dose-related trend of reduced mean brain weight which was significant at a dose rate of 300 millirads/day. Pb significantly reduced relative brain weight at both levels whereas combined exposure to HTO and Pb produced an interaction effect which was less than either agent alone.

One of the consequences of chronic Pb exposure is shown in the organ Pb levels in Table III. HTO had no effect on Pb accumulation and all data have been compiled by Pb treatment. As expected, the major repositories of Pb were bone, kidney and liver. Of particular interest were the observations that a) the maternal:neonatal blood Pb ratio was a constant ratio of approximately 1.3 across all Pb groups; b) intrauterine exposures resulted in Pb accumulation in neonatal lung but not brain; c) there appeared to be a sex difference in Pb content of adult organs.

Postnatal brain catecholamine levels are presented in Table IV. The relative brain weight deficits present at birth in the F_2 due to HTO or Pb exposures were not present at 28 or 180 days. Neither HTO, HTO/Pb nor generation affected NE or DA concentrations in whole brain and the results have been combined by Pb exposure for both generations. NE and DA were both affected by 50 ppm; at the earlier age DA was significantly depressed and in the adult the NE significantly increased.

The effects of HTO and/or Pb on CNS development are presented in Tables V-VII. There were no significant differences in data obtained from the F_1 and F_2 generations so the results have been combined. HTO or Pb and the interaction of HTO/Pb tended to delay the development of the reflexes and the age at eye opening. Statistically significant delays occurred with the righting reflex (Table VII) and eye opening (Table VI) at a dose rate as low as 3 millirads/day and with the startle reflex at 300 millirads/day. Righting reflex development was delayed after 5 ppm Pb exposure. In nearly all cases the interactions of HTO and Pb were less than additive in their effect.

Table III

Lead Concentrations in Organs ($\mu\text{g}/100\text{g}$ wet weight)^a

Pb Level (ppm)	F ₁ Dams			F ₂ Neonates			180 Day Males (F ₁ + F ₂)		
	0	5	50	0	5	50	0	5	50
Blood	(34) ^b 11±0.5 ^c	(34) 13±0.5	(37) ^e 26±1	(26) 8±0.6	(31) 11±0.5 ^e	(29) 20±1 ^e	(43) 5±0.5	(48) 6±0.4	(49) ^d 10±0.6 ^d
Bone							(35) 115±14	(48) ^e 315±15 ^e	(39) 1410±82 ^e
Kidney	(34) 15±0.7	(34) ^e 44±2	(33) 176±8 ^e	(25) 16±1	(23) 20±1 ^d	(22) 20±1 ^d	(41) 18±0.8	(49) ^e 41±2 ^e	(43) 102±6 ^e
Liver	(35) 11±0.5	(36) 18±0.7 ^e	(26) 73±7 ^e	(27) 8±0.8	(25) 9±0.4	(20) 24±2 ^e	(40) 18±1	(46) 18±0.9	(42) 22±0.9 ^e
Brain				(21) 10±0.9	(23) 10±0.6	(19) 10±0.9	(20) 18±1	(21) 20±2	(23) 27±2 ^e
Lung				(23) 19±1	(24) 29±2 ^e	(21) 29±3 ^e			
Heart				(18) 10±1	(19) 7±0.5	(21) 8±0.7			
Spleen				(17) 15±2	(21) 20±2	(21) 18±2			
Testes							(43) 18±0.8	(49) 18±0.9	(47) 18±1.0

a = No effect of tritium exposure. All tritium groups were pooled by Pb treatment.

b = sample number

d = p<0.05.

c = mean ± SE

e = p<0.01.

Table IV

Effects of Pb Exposure on NE and DA Concentrations in Whole Rat Brain^a (ng/g)

Pb Level	28 days old		180 days old	
	NE	DA	NE	DA
0 ppm	(50) ^b 362±13 ^c	(53) 572±22	(56) 465±12	(56) 890±25
5 ppm	(56) 384±11	(56) 526±20	(59) 455±14	(64) 901±26
50 ppm	(55) 358±11	(52) 453±19 ^e	(43) 505±15 ^d	(42) 917±42

a = male rats; F₁ and F₂ generations combined; tritium data pooled by Pb exposure.

b = sample number

c = mean ± SE

d = p<0.05 by ANOVA and t-test

e = p<0.01 by ANOVA and t-test

Table V

Age at Development of the Startle Response in Rats Exposed to HTO and/or Pb^{a,b}

Treatment	0.0 μ Ci/ml	0.01 μ Ci/ml	0.1 μ Ci/ml	1.0 μ Ci/ml
0 ppm	(11) ^c 11.8 \pm 0.2	(13) 12.4 \pm 0.3	(13) 12.3 \pm 0.2	(5) 13.0 \pm 0.4 ^d
5 ppm	(9) 12.0 \pm 0.2	(10) 11.8 \pm 0.2	(12) 12.8 \pm 0.3 ^d	(11) 12.1 \pm 0.2
50 ppm	(10) 12.4 \pm 0.2	(9) 12.4 \pm 0.4	(12) 12.4 \pm 0.2	(12) 12.2 \pm 0.3

Table VI

Age at Eye Opening in Rats Exposed to HTO and/or Pb^{a,b}

Treatment	0.0 μ Ci/ml	0.01 μ Ci/ml	0.1 μ Ci/ml	1.0 μ Ci/ml
0 ppm	(11) ^c 15.2 \pm 0.2	(13) 16.0 \pm 0.1 ^d	(13) 15.9 \pm 0.2 ^d	(5) 16.3 \pm 0.2 ^d
5 ppm	(9) 15.4 \pm 0.2	(10) 15.5 \pm 0.3	(12) 16.5 \pm 0.3 ^d	(11) 15.4 \pm 0.2
50 ppm	(10) 15.8 \pm 0.2 ^d	(10) 15.3 \pm 0.2	(11) 16.2 \pm 0.3 ^d	(12) 15.3 \pm 0.2

Table VII

Age at Development of the Righting Reflex in Rats Exposed to HTO and/or Pb^{a,b}

Treatment	0.0 μ Ci/ml	0.01 μ Ci/ml	0.1 μ Ci/ml	1.0 μ Ci/ml
0 ppm	(11) ^c 17.7 \pm 0.2	(13) 19.6 \pm 0.5 ^d	(13) 18.9 \pm 0.2 ^d	(5) 20.0 \pm 0.4 ^d
5 ppm	(9) 18.8 \pm 0.4 ^d	(10) 18.7 \pm 0.2 ^d	(11) 19.5 \pm 0.2 ^d	(11) 18.8 \pm 0.4 ^d
50 ppm	(10) 19.8 \pm 0.7 ^d	(9) 19.2 \pm 0.5 ^d	(12) 19.4 \pm 0.4 ^d	(12) 19.5 \pm 0.3 ^d

a = Interaction of HTO and Pb was significant by two-way ANOVA

b = Values given are for mean day of development \pm SE. Value in parenthesis is the number of F₁ + F₂ litters (5 animals/litter).

c = sample number

d = p<0.05 by ANOVA and t-test

Table VIII

Residential Maze Activity of Adult Male Rats Exposed to HTO and/or Pb^{a,b} (counts/hour)

Treatment	0.0 μ Ci/ml	0.01 μ Ci/ml	0.10 μ Ci/ml	1.0 μ Ci/ml
0 ppm	263 \pm 10	224 \pm 20	218 \pm 7 ^c	177 \pm 10 ^c
5 ppm	177 \pm 15 ^c	215 \pm 20	203 \pm 30	187 \pm 12 ^c
50 ppm	199 \pm 32	196 \pm 19 ^c	194 \pm 17 ^c	207 \pm 18 ^c

a = Interaction of HTO and Pb was significant by two-way ANOVA.

b = 180 days old; F₁ and F₂ generations combined; n = 5 litters per dose group; each litter represented by 3 rats, mean \pm S.E.

c = p<0.05 by ANOVA and t-test.

Table IX

Interval Analysis of the Anesthetized Electroencephalogram of F_1 Adult Male Rats Exposed to HTO and/or Pb							
TREATMENT	HTO only ($\mu\text{Ci/ml}$)				Pb only (ppm)		PPb/HTO
	0.0	0.01	0.10	1.0	5	50	50/1.0
	(5) ^a 23.8 \pm 3.7 ^b	(5) 24.3 \pm 3.0	(5) 17.9 \pm 2.1	(3) 14.6 \pm 0.9	(5) 18.5 \pm 1.8	(5) 25.4 \pm 3.4	(6) 24.4 \pm 3.7
ZPC/5 sec.							
% Beta	16.7 \pm 3.5	16.6 \pm 3.7	10.0 \pm 3.1	7.4 \pm 1.2	8.3 \pm 8.5	14.8 \pm 3.7	16.8 \pm 4.3
% Alpha	20.5 \pm 3.1	20.1 \pm 1.7	14.3 \pm 1.7	13.3 \pm 0.8	13.9 \pm 2.2	17.2 \pm 2.2	18.6 \pm 2.5
% Theta	28.4 \pm 4.0	31.8 \pm 2.5	27.2 \pm 4.2	24.7 \pm 4.8	32.8 \pm 3.0	31.4 \pm 4.9	33.4 \pm 3.1
% Delta	33.4 \pm 8.3	31.8 \pm 6.4	46.9 \pm 6.2	49.5 \pm 2.0	45.0 \pm 5.7	30.9 \pm 5.7	31.9 \pm 6.8

a = sample number

b = mean \pm SE

The effects of the various HTO and/or Pb exposure regimens on the spontaneous locomotor activity of 165-day-old male rats are presented in Table VIII. Again no consistent differences were observed between generations and the data have been combined. The values presented are the mean counts per hour per day for 3 littermates over 3 consecutive days of residence in the maze. A total of 5 litters ($F_1 + F_2$) represent each dose group. HTO exposure produced a dose-related hypoactivity which was significant at 30 millirads/day. Pb exposure at 5 ppm produced results similar to 300 millirads/day but no statistically significant effect on activity was produced by the highest dose of Pb. In all cases, combined exposure to HTO/Pb was less than the additive effects of either individual agent.

In Table IX the interval analysis of the EEG recordings from adult F_1 males is presented. No interaction between HTO and Pb emerged from a two-way ANOVA of the EEG parameters so that only the values for HTO, Pb and the highest HTO/Pb exposures are given. Zero potential crossover (ZPC) is the total number of waveforms per 5-second epoch. The % β , α , θ and δ represent the relative occurrence of specific frequencies of electrical activity within the 5-second epoch. Although not statistically significant, HTO exposure tended to decrease ZPC rate as did the 5-ppm Pb exposure but 50 ppm Pb and the combined HTO/Pb rates were similar to control levels. The reduction in ZPC rate occurred as a result of decreases in the % abundance of the α and β frequency classes with a corresponding increase in the δ frequency. However, the EEG data from the F_2 rats provided no indication of treatment-related alterations in any parameter of the EEG interval histograms.

Complete gross and histopathologic evaluation of F_1 and F_2 adult male rats, exposed to HTO and/or Pb for approximately 200 days, showed no evidence of pathologic sequelae at the dose levels employed.

DISCUSSION

P_0 rats were pre-treated with Pb for 40 days prior to mating to establish steady-state levels in the major soft-tissue repositories for Pb since, in humans, soft-tissue concentrations of Pb are relatively constant after the second decade of exposure [16]. In rats, the liver and kidney constitute rapid exchange pools for Pb with biological half-lives of approximately 4-6 days [17].

Assuming an average water consumption of 25 ml/day, the total daily Pb intake of rats in this experiment was approximately 0.15-1.5 mg or 0.6-6 mg/kg/day. Human adult daily intake averages 0.4 mg or 0.006 mg/kg/day [18].

On the basis of previous work [3], the HTO activities employed would not be expected to produce effects evident on the F_1 generation at birth.

Similarly, continuous feeding of 64 or 512 ppm Pb in the diet of P_0 rats has been reported to have no effect on F_1 litter size or body weight [19].

Throughout the exposure of the F_1 and F_2 generations no overt signs of acute toxicity were noted with any treatment except for a transient body-weight deficit in F_2 HTO animals from days 9-15 postnatally. Elevated urinary levels of Δ ALA, which is considered to be a sensitive indicator of Pb intoxication [20], were seen in the 50 ppm F_1 adult rats at approximately 90 days of age.

The reproductive performance of the F_1 generation exposed to HTO was consistent with an earlier report [21] which showed that continuous parental exposure to activities up to 1.0 μ Ci/ml had no effect on litter size, resorptions, pre-implantation deaths or neonatal weight.

The effects of lifetime parental exposure to relatively low levels of Pb on the F_2 has been investigated by Morris et al. [19] and Schroeder and Mitchener [22]. The former found no effects on litter size or neonatal weight at 64 or 512 ppm. Schroeder and Mitchener's study is pertinent to the present investigation. They exposed an F_1 generation of rats from conception to 25 ppm Pb and examined the reproductive effects. However, the manner of presentation of the results makes comparisons with the present study impossible. Nevertheless, we noted a significant increase in F_2 litter size in the 5-ppm group coincident with an increased corpora lutea (CL) count. At 50 ppm, no difference from control was seen in litter size, probably because the increased CL was offset by increased pre-implantation deaths.

The decreased relative brain weight of F_2 neonates produced by parents with lifetime exposures to 300 millirads/day confirms a previously observed HTO effect [21]. With respect to Pb, we observed a dose-independent decrease in the relative brain weight at birth which has not previously been reported for chronic low-level exposures. At later postnatal ages this effect was not observed in any treatment group. No topographical or internal organ anomalies were evident in any dose group at birth.

The blood Pb levels in F_1 mothers and their offspring serve to support Baltrop's suggestion that a maternal-fetal blood Pb equilibrium exists [2]. Interestingly, his study of humans shows an average maternal-fetal blood Pb ratio of 1.35, nearly the same value observed in our rats. The differences in blood and organ Pb levels between adult males and F_1 dams is attributable in part to the greater daily water consumption of the female rat [23].

The results of reflex testing indicate that both Pb and HTO administration delay CNS development. A dose-related delay in the righting reflex was produced by Pb with no observed effect on growth. This suggests that Pb is acting directly on the nervous system. HTO produced a delay in the development of the righting reflex and eye opening at all exposure levels and in the startle response at the 1.0 μ Ci/ml level.

The transient depression of body weight which occurred during the testing period for F_2 startle reflex and eye opening may have contributed to these results since undernutrition may be a factor in the observed delays [24]. However, these effects had disappeared by day 16 which precedes the development of the righting reflex.

The ANOVA showed a significant interaction between Pb and HTO on reflex development and eye opening, indicating the effects are not additive. By inspection, the interactions are less than additive in all cases.

Locomotor activity in the adult animals was also affected by Pb and/or HTO. The HTO administration produced a dose-related decrease in the total activity whereas Pb exposure produced depressions of activity unrelated to dose. As with the reflex development, the effects on activity produced by combined exposure to Pb and HTO were less than additive.

Since the adult CNS has been shown to be very insensitive to both Pb [25] and ionizing radiation [26], these behavioral differences seen in the adult animals are probably due to the effects of Pb and/or HTO exposure on the CNS either in utero or during early development.

Brain catecholamines are known to be associated with spontaneous locomotor activity [27]. The slight, but statistically significant increase in whole-brain NE concentration and the recovery of DA concentrations in the 50-ppm treated adults was not the expected result for hypoactive animals. An explanation for this apparent conflict is that whole-brain catecholamine assays may mask regional effects which are directly involved in the expressed behavior.

The results of the adult F_1 EEG analyses are compatible with the hypoactivity responses of most treatment groups. High-voltage slow waves are characteristic of both natural and anaesthetized sleep. Thus, the increased % abundance of the δ waves is suggestive of neuronal synchronization and a "resting" CNS. Unfortunately, the significance of these data and the correlation are open to question because of the inability to reproduce these findings in the F_2 generation.

The objective of this study was to determine if 2 potentially synergistic environmental pollutants would in fact interact and to assess the impact on population exposure standards. For the conditions of our study, we have concluded that combined exposures to HTO and Pb are less than additive in their effect and do not impact on the population exposure standards for either agent. The principal findings of this work were that continuous, low-level exposures to 3 millirads/day of HTO or 5 ppm Pb can produce biologically important consequences to mammals.

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DISCUSSION

C.A. SONDHHAUS: Your data on age of onset of eye opening and startle response suggest an antagonistic effect of HTO and Pb together, which appears to cancel the delay due to either of them alone. But does "loss of additivity" necessarily indicate that either agent "protects" against the other? Perhaps the respective insults simply mask each other in different ways. Could you please comment on any such indications or possible mechanisms?

D.F. CAHILL: We do not think that "loss of additivity" should necessarily be equated with a protective effect of either agent against the other. Since the exposure to HTO or Pb alone were sufficient to produce effects, whereas the combined exposures were less effective than either agent, we could speculate that the low level of insult produced by either was insufficient to provoke a compensatory response but that the combined insults did trigger a compensatory reaction.

Y. NISHIWAKI: When we were trying to use deuterated water (heavy water) for the diagnosis of kidney disease 20 - 30 years ago in Japan, we observed that the water metabolism and the size of the water pool as well as the excretion rate of water were affected in cases of disturbance of renal function. Is there any possibility in your experiment that the administration of lead could have affected renal function, and that the water metabolism, the size of the water pool of the body and the excretion rate of tritiated water were changed?

D.F. CAHILL: Experiments by Goyer at the University of North Carolina have shown that exposure to high levels of lead, of the order of 1 000 to 10 000 ppm, will produce renal dysfunction with a characteristic occurrence of nuclear inclusion bodies in renal tubular cells. Our histopathologic evaluations of the kidneys of rats exposed to much lower doses of lead showed no evidence of nuclear inclusion bodies. This would imply that renal damage did not occur. However, we did not directly test for renal function.

Our experimental design would not permit us to determine any fluctuations in the body water pool because the animals were on a continuous regimen of tritiated drinking water at a constant specific activity; nor was the urinary excretion rate measured.

R.B. HOLTZMAN: What were the lead concentrations in the animals, and how do they compare with those in man and in unexposed animals?

D.F. CAHILL: The lead concentrations in a number of neonatal and adult organs as a function of the exposure regimen are presented in Table III. With respect to the unexposed animals, the chronically exposed adult rats showed significantly higher lead levels in the major organ repositories even at the 5 ppm exposure level.

The blood lead levels of F_1 dams and F_2 neonatal rats exposed to 50 ppm correspond roughly to the average level of lead in human blood. Since at our lowest exposure level we were providing approximately 100 times the adult human daily intake in terms of mg of lead per kg of body weight, the lead levels in the rat organs were many times those of humans.

E.E. POCHIN: If you test a large number of deviations for statistical significance, some, e.g. 5%, will exceed the $p = 0.05$ level purely by chance. Are your estimates of significance corrected for this?

D.F. CAHILL: If I interpret your question correctly, you are asking whether in a 12-cell matrix we would expect one observation to exceed $p < 0.05$ purely by chance. Yes, we would, but similarly we would also expect that the "significant" observation would occur at random throughout the eleven treatment cells. The clear dose-responses in a number of parameters which we measured would argue against these being chance occurrences. The absence of clear dose-responses to HTO in several cases is probably a function of the extremely low radiation doses employed and the insensitivity of the functional tests for discriminating between the low-level effects.

A one-way ANOVA was applied to the results with only HTO or only lead and a two-way ANOVA to the data from groups exposed to both HTO and lead. This was used to screen for differences between treated and untreated populations. The doses at which significant differences arose were further defined by the t-test.

H. GLUBRECHT: I should like to make a general comment which relates to nearly all the papers presented at this session. I wonder if it is meaningful, while investigating incorporated radioisotopes, to make complicated and sometimes very arbitrary calculations in order to obtain dose values in rads. We know that, at least in some cases, the transmutation effect plays a major role as compared to radiation effects. Moreover, even if radiation alone is important, all effects depend on the distribution of the radionuclides in the molecular structure of the cells and organisms and this is true especially in the case of tritium with electrons of about 1 μ m range. This is a biochemical problem and therefore cannot be solved by

microdosimetry. In cases where we do not have a fairly homogeneous distribution, I would suggest that we desist from giving absorbed energy dose values and define the experimental situation only by parameters which we know with certainty, such as the specific activity of the radioisotope administered and the chemical and physical parameters of treatment.

CURRENT STATUS OF UTAH LONG-TERM ^{239}Pu STUDIES*

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Abstract

CURRENT STATUS OF UTAH LONG-TERM ^{239}Pu STUDIES.

Osteosarcomas have been the chief cause of death in the Utah young adult beagles injected intravenously with ^{239}Pu citrate solution. There was nearly a 100% incidence of bone sarcomas above the 0.015 $\mu\text{Ci/kg}$ dose levels. At the 0.015 $\mu\text{Ci/kg}$ dose level there was a 29% (4 of 14) incidence. The latent period lengthened with decreasing injected dose. The only other pathology was liver regenerative foci occurring at doses as low as 0.0018 $\mu\text{Ci/kg}$ and an incidence of 12% (3 of 25) bile duct carcinomas at the 0.048 and 0.015 $\mu\text{Ci/kg}$ dose levels. Studies using older (5-year) and growing (3-months) beagles, young adult St. Bernards and young adult beagles injected with polymeric ^{239}Pu showed variation in sensitivity for bone tumour induction. The polymeric model represents a continuous cycling of ^{239}Pu from the liver to a mature skeleton and the St. Bernard model typifies the relationship between high spontaneous and radiation-induced tumour sensitivity. No results are available for the older adult, but one bone tumour in a growing beagle injected with 0.3 $\mu\text{Ci/kg}$ suggests decreased sensitivity. There is increased sensitivity in St. Bernards and polymeric injected dogs for bone tumour induction.

1. INTRODUCTION

Studies of the long-term biological consequences of internally deposited radioactive elements are unique endeavours. The cost in terms of manpower, time and funds discourages even countries with virtually unlimited resources from launching such projects. Yet these studies are necessary. The former Division of Biology and Medicine of the US Atomic Energy Commission, now the US Energy Research and Development Administration, is to be congratulated for their imagination and courage in initiating these long-term experiments involving internally deposited radionuclides in animals. These experiments now serve as models and standards for studying the long-term effects of any toxic substance.

Shields Warren, John Bowers, Charles Dunham, Paul Pearson, Walter Claus, Robley Evans, Wright Langham and Austin Brues developed the idea in 1950 that an experimental analogy tied to the maximum permissible level of ^{226}Ra in man would make it possible to predict the long-term toxicity of plutonium and other radionuclides in man [1-5]. Specifically,

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from the beagle studies involving ^{226}Ra and ^{239}Pu , the following relationship can be used to predict the toxicity of ^{239}Pu in man:

$$\frac{\text{Beagle } ^{239}\text{Pu toxicity}}{\text{Beagle } ^{226}\text{Ra toxicity}} \approx \frac{\text{Man } ^{239}\text{Pu toxicity}}{\text{Man } ^{226}\text{Ra toxicity}}$$

Subsequent complementary chronic animal experiments were developed at Hanford, Davis, Albuquerque and Argonne.

1.1. Experiments with internally deposited radionuclides

Investigators who study the toxicity of internally deposited radionuclides in animals to predict the toxicity in man find such projects extremely challenging. Internally deposited radionuclides are known to be very selective in their distribution. They may seek out only one tissue, organ or organ system. Their initial distribution is usually quite non-uniform and characteristically changes with time both in concentration and deposition pattern. Examples of ^{239}Pu distribution in liver and skeleton are given further on. In addition, investigators must be students of the critical organ or tissue at risk for both animal and man in order to predict intelligently the expected toxicity in man.

This short report on the Utah studies should provide an adequate understanding of the complexity of such studies. Finally, since there are so few studies because of their high cost, it is the duty of investigators in this field to report clearly any available data so that others can make use of them.

1.2. Experimental design of Utah studies

In the early 1950s, scientific consultants helped to establish the dose levels and the number of dogs at each dose level. Lower dose levels were added in 1963 because an osteosarcoma occurred in a beagle at the then lowest dose level of ^{239}Pu (0.015 $\mu\text{Ci/kg}$).

Briefly, young adult beagles are put into the toxicity study at graded injection levels. Each animal receives the designated dose of one radionuclide in a single intravenous injection at approximately 17 months of age. At this age the skeleton is mature with all epiphyses fused except those of the ribs. Twelve such groups have been injected for each of the six radionuclides: ^{226}Ra , ^{239}Pu , ^{228}Ra , ^{228}Th , ^{90}Sr and ^{241}Am . The present injection programme includes ^{226}Ra and ^{239}Pu at lower dose levels, ^{241}Am , ^{249}Cf , and ^{252}Cf .

The dose levels used are based on a scheme which uses ten times the maximum permissible concentration of ^{226}Ra in man, designated level 1:

$$\text{Level 1} = \frac{10 \times 0.1 \mu\text{Ci } ^{226}\text{Ra}}{70 \text{ kg}} = 0.0143 \text{ "retained" } \mu\text{Ci/kg body weight.}$$

All other injection levels are simple multiples of level 1 (Table I).

1.3. Critical tissues

The skeleton, liver, thyroid, kidney and eye can be organs of high concentration (Table II). The internally deposited radioactivity has produced

Table 1. Utah Beagles Dose Levels

Level	0.1	is	1/27	of	level 1
"	0.2	"	1/9	"	"
"	0.5	"	1/3	"	"
"	0.7	"	2/3	"	"
"	1.5	"	2	times	"
"	1.7	"	3	"	"
"	2	"	6	"	"
"	3	"	18	"	"
"	4	"	54	"	"
"	4.5	"	94	"	"
"	5	"	162	"	"

Table II. Tissues With High Concentration of the Radionuclide Studied

Nuclide	Half-period (yr)	Radiation*	Tissue with High Concentration
²⁵² Cf	2.65	α, f	Liver, skeleton, thyroid, kidney
²⁴⁹ Cf	352	α	Liver, skeleton, thyroid, kidney
²⁴¹ Am	433	α	Liver, thyroid, skeleton, kidney
²³⁹ Pu	24400	α	Liver, skeleton
²²⁸ Th	1.91	α	Skeleton, kidney
²²⁸ Ra	5.77	β, α	Skeleton, eye
²²⁶ Ra	1600	α	Skeleton, eye

*Cf decays by spontaneous fission in about 3% of its disintegrations, but its energetic fission fragments account for about half of the tissue dose. ²²⁸Ra decays by β emission, but most of the dose results from build-up of its α -emitting decay products.

bone sarcomas, head sinus carcinomas, liver tumours and eye melanomas [6-11]. Bone sarcomas have been the most important delayed effect in our colony. This is especially significant because the beagle has an extremely low natural incidence of osteosarcoma; only one case per 100 000 long-lived beagles [12,13]. Recently we have initiated studies with the St. Bernard, a breed with a high natural incidence of bone sarcoma, to find whether they are more susceptible to radiation-induced osteosarcoma.

2. RESULTS

It is too soon to discuss the low-dose effects of ²⁴¹Am, ²⁴⁹Cf and ²⁵²Cf;¹ therefore only our exciting ²³⁹Pu studies are summarized here:

¹ The current status of these experiments is summarized in our most recent Progress Report COO-119-250. (Copies are available upon request).

- (a) Distribution in liver
- (b) Liver lesions
- (c) Distribution of bone in young adult beagles
- (d) Skeletal toxicity in young adult beagles
- (e) Skeletal toxicity in adult beagles
- (f) Skeletal toxicity in rapidly growing beagles
- (g) Skeletal toxicity as a function of age
- (h) Skeletal toxicity in St. Bernards
- (i) Skeletal toxicity of polymeric ^{239}Pu in beagles

2.1. ^{239}Pu distribution in liver

In our lower-level ^{239}Pu beagles, the net liver burden remains roughly constant at about 30% of the injected activity for about the first 1000 days after injection [6]. After that, the liver burden decreases with a half-time of about 3000 days.

The initial distribution of ^{239}Pu is rather uniform throughout the liver following intravenous injection of monomeric ^{239}Pu in citrate solution. For several hundred days most of the ^{239}Pu is retained in the hepatic cells, but subsequently a high percentage is translocated into the liver reticuloendothelial cells. This movement to the reticuloendothelial cells occurred with progressively longer latent periods as the injected dose is decreased. Thus, the distribution within the liver changes from being rather uniform to extremely non-uniform.

2.2. Liver lesions

The liver lesions have consisted principally of hepatic cell necrosis followed by regenerative changes [11]. Significant regenerative changes were produced at injected doses extending down to $0.0018 \mu\text{Ci/kg}$. The restoration of the lost cells was sufficient to maintain normal liver weights at the low dose levels.

2.2.1. Bile duct carcinoma

Intrahepatic bile duct tumours have been observed in about 12% of the dogs (3 of 25) surviving beyond 3000 days with doses of ^{239}Pu below the 0.048 Ci/kg levels and an average liver dose is less than 220 rads. Other forms of primary liver neoplasia have not been observed (Table III).

2.2.2. Effect of age

Surprisingly, only subtle liver changes were observed in three-month-old beagles injected at our highest dose level ($2.7 \mu\text{Ci/kg}$). Currently no adult beagle (5-year-old) data are available. (Taylor, G.N., personal communication).

2.2.3. Relationship to man

Unfortunately, little effort has been made to evaluate the risk to man from ^{239}Pu -induced liver tumours. The ICRP task group on metabolism of compounds of plutonium and other actinides concluded that for Pu reaching the blood from the thoracic region, 45% would deposit in the liver and 45% in

Table III. Incidence and Average Age at Recognition of Malignant Bile Duct Carcinoma in Dogs Given the 0.048 and 0.015 $\mu\text{Ci } ^{239}\text{Pu/kg}$

	Controls	^{239}Pu	^{226}Ra
Dogs at risk	98	25	12
No. of tumors	0	3*	0
% Incidence	0	12.0	0
At Recognition:			
Average age	-	4144	-
Range of age	-	(3376-5257)	-

*Average liver dose was approximately 100 and 225 rads respectively.

the skeleton, with the remaining 10% deposited in other tissues or excreted. Thus, the average organ dose rate is ~4 times higher in the blood-filled liver than in the marrow-free skeleton of standard man. Other studies show the retention by the liver to be even higher [14-16].

Mays et al. [17] speculated that environmentally and occupationally acquired plutonium in humans may induce twice as many liver tumours as bone sarcomas. However, this estimate is based on the uncertain assumption that the ratio of liver tumour induction per liver rad to bone sarcoma induction per average skeletal rad is equal in man and dog. It would be much safer to tie into the human experience with α -particle induced liver tumours.

About 200 fatal liver tumours have occurred among some 4000 European Thorotrast patients under study. Correcting for self-absorption of energy within the Thorotrast aggregates, the typical intravascular injection of about 25 mlitres of Thorotrast produces a dose rate of about 25 rad/a to the human liver, or a liver dose of about 500 rads in 20 years. While it is tempting to use this direct human toxicity information to calculate a risk coefficient for Pu-induced liver tumours in man (5% incidence/500 rad = 100 liver tumours/ 10^6 person·rad), the question has been raised as to how much of the liver toxicity from Thorotrast is due to the chemical toxicity of the ThO_2 , and how much due to the radiation [18]. This question could be resolved by comparing the effects of Thorotrast (radiation plus chemical?) with Pu (radiation alone) in beagles. The task is greatly simplified because we already have data in hand on the toxicity of ^{239}Pu in the liver, and thus additional ^{239}Pu -injected beagles would not be required. Stated in equation form:

$$\frac{\text{Man Pu liver toxicity (unknown)}}{\text{Man Thorotrast toxicity (known)}} = \frac{\text{Beagle Pu liver toxicity (known)}}{\text{Beagle Thorotrast toxicity (needed)}}$$

To gain a better idea of liver tumour risk in man, only new information on the Thorotrast toxicity in the beagle would be needed to solve the above equation for the predicted toxicity of Pu to the human liver. (Mays, C.W., Taylor, G.N., personal communication).

Table IV. Age Factors Influencing the Magnitude of ^{239}Pu Insult to Bone in Beagles of Various Ages*

Bone Surface	Rapidly Growing (3 months)	Young Adult (1.7 yr)	Adult (5 yr)
A. Total quantity of surface	low	maximum	maximum
B. Forming surfaces**	> 50%	< 50%	~ 5%
C. Resorbing surfaces**	< 50%	< 50%	~ 1%
D. Resting surfaces**	0	~ 20%	~ 95%
E. Apposition (burial)	~ 2 $\mu\text{m/d}$	~ 1.5 $\mu\text{m/d}$	~ 1 $\mu\text{m/d}$
F. Number of sensitive cells	large	moderate	extremely small
G. Residence time of sensitive cells	brief	moderate	prolonged
H. Residence time of initial ^{239}Pu	very brief	brief	prolonged
I. Concentration of ^{239}Pu at 200 days post-injection	low	moderate	extremely high
Magnitude of insult [†] (F + G + H + I)	low	moderate	high

*Assumes the initial ^{239}Pu deposited on bone surfaces and the subsequent ^{239}Pu circulating in blood are equal regardless of age.

**Values listed for B, C, D are very rough estimates.

[†]The magnitude of the insult is the sum of the number of cells at risk, residence time, and concentration of ^{239}Pu on bone surface.

2.3. Distribution of bone in young adult beagles (17 months old)

The distribution of ^{239}Pu in young adult beagles was similar to the classic studies of Arnold and Jee using the rat [19]. The initial deposition of ^{239}Pu in the skeleton is on bone surfaces when the monomeric form is injected intravenously. Soon after, many of the bone deposits are quickly buried by the apposition (formation) of new bone tissue or are recycled by resorption of old bone. Some of the resorbed radionuclide eventually recycles back to bone surfaces where it is again subjected to burial or resorption. Some of the ^{239}Pu released by the liver which reaches the blood is redeposited on bone surfaces. As time progresses, the pattern changes from a surface deposition, in which about half of the emitted α particles penetrate into cellular regions, towards a volume deposition, in which most of the α particle energy is absorbed within bone mineral rather than in living cells [20,21].

2.3.1. Effect of age upon distribution patterns

Table IV summarizes the influence of age upon the distribution of ^{239}Pu , assuming the initial uptake by bone surfaces is equal regardless of age. In rapidly growing skeletons, most of the initial deposits of ^{239}Pu on bone surfaces are rapidly buried, and the rest is resorbed. Some of the recycled ^{239}Pu from the bone and liver will be deposited on new bone surfaces and these less radioactive bone surface deposits in turn will be rapidly buried or resorbed. The net result is a short residence time for bone surface ^{239}Pu in rapidly growing animals. In adults, the residence time of ^{239}Pu on bone surfaces is prolonged due to slower skeletal turn-over. After the initial surface deposits, the ^{239}Pu from bone and liver finds its way to bone surfaces already laden with ^{239}Pu which have not been remodelled. Not only is there a prolonged ^{239}Pu bone surface residence time in adult bone, but they accumulate more ^{239}Pu with time.

2.3.2. Cells at risk or the sensitive cellular volume

Any cell type within the field of irradiation must be considered as a potential precursor of a tumour [22-24]. Recently Hashimoto and Jee [25], using the criteria of: (1) within range of an α particle; (2) proven inclination toward osteogenesis; (3) capability of rapid proliferation; and (4) morphological similarity to tumour cells, postulated the strongest choice to be the osteoprogenitor cell. The osteoblast and reticular cell of the marrow were considered slightly less likely. On the contrary, Marshall and Groer postulate it to be the endosteal cells (resting osteoblast, bone lining cells) located on bone surfaces as the cell at risk and have neglected to deal with other cells exposed to the range of the α particle [26].

The numbers of osteoprogenitor cells and osteoblasts diminish with age. The largest reduction is found between the young adult and the aged. Values for the distribution of reticular cells are not available, but one is safe to say that their numbers diminish with age.

2.4. Skeletal toxicity in young adult beagles

The bulk of our efforts have been with the young adult beagle (Table V). Bone sarcomas have been the important long-term effect. There was nearly a 100% incidence above the $0.048 \mu\text{Ci/kg}$ injected dose levels. At the $0.015 \mu\text{Ci/kg}$ dose level, four out of 14 dogs succumbed to bone sarcomas. Most of the dogs from four lower dose levels (0.01 , 0.005 , 0.0018 and $0.006 \mu\text{Ci/kg}$) are still alive (Nov. 1975). In general, the appearance time of tumours lengthened with decreasing injected dose.

In the lower dose levels, initially about 50% of the injected activity is found in the skeleton. After that, the skeletal burden decreases to about 20% at 12 years post injection [6].

Initially the bones of these dogs are rapidly growing and after about six months they mature and behave like adult bones. This is the case when one uses the lumbar vertebra as a typical model. Initially there is a large population of cells near bone surfaces followed later by a paucity of cells. Therefore, both the distribution of ^{239}Pu and the mapping of the cells at risk during the lifespan of these dogs is complex, and more detailed studies are needed.

Table V. Current Status of Pu-239/Toxicity/Young Adult Beagle Study*

Code	Dose $\mu\text{Ci/kg}$	Alive	Dead	Post-injection months (alive)	Number of Dogs		
					bone tumor	sinus carcinoma	bile duct carcinoma
P0	0	34	16	36-135	0	0	0
P0.1	0.006	26	2	20-135	0	0	0
P0.2	0.0018	41	5	20-132	0	0	0
P0.5	0.005	36	2	13-135	0	0	0
P0.7	0.010	38	0	13-69	0	0	0
P1	0.015	11	14	20-69	4(121 \pm 35) [†]	0	1
P1.7	0.048	0	13	**	12(106 \pm 21)	0	2
P2	0.095	0	12	**	10(87 \pm 17)	1	0
P3	0.300	0	12	**	12(55 \pm 7)	0	0
P4	0.900	0	12	**	12(45 \pm 7)	0	0
P5	2.700	0	9	**	8(48 \pm 10)	0	0

*1) Regenerative foci in liver apparent at doses P0.2 through P5.

2) Hematologic neoplasia insignificant.

3) Tooth loss significantly greater in levels P1.7 through P5.

** No dogs currently living.

[†] Latent period in months \pm S.D.

Surprisingly, head sinus carcinomas were rare in the Utah beagles, with only one observed case (0.095 $\mu\text{Ci } ^{239}\text{Pu/kg}$). None has been observed in our ^{226}Ra dogs (Table VI). This is in contrast to the human ^{226}Ra and ^{228}Ra cases for which about 27 head sinus carcinomas and 54 bone sarcomas have been evaluated [27-31]. One could speculate that human sinuses are subjected to recurring infection, a possible factor contributing to carcinoma induction. The beagles seldom contract sinus infection (Taylor, G.N., personal communication). But on the other hand, no head sinus carcinomas have yet appeared in the German ^{224}Ra patients although 54 bone sarcomas have been recorded (Spiess, H., Mays, C.W., personal communications).

The average skeletal dose for ^{239}Pu and ^{226}Ra for the Utah beagles has been well worked out from the retention data. C.W. Mays has estimated the RBE of $^{239}\text{Pu/Ra}$ is 16 in beagles using average skeletal doses and the linear model. His RBE for man is 4 to 33. See the discussion of his result in paper IAEA-SM-202/806, these Proceedings.

Table VI. Current Status of Ra-226/Toxicity/Young Adult Beagle Study*

Code	Dose $\mu\text{Ci/kg}$	Alive	Dead	Post injection months (alive)	No. of dogs with osteo- sarcoma (latent period \pm S.D. in months)	No. of dogs with sinus carcinoma	No of dogs with eye melanomas
R0	0	32	12	53-131	0	0	0
R0.2	0.007	9	1	95-131	0	0	0
R0.5	0.02	24	1	98-124	0	0	0
R1	0.06	7	15	95-118	0	0	1
R1.7	0.16	0	14	**	1(183)	0	5
R2	0.35	0	13	**	6(128 \pm 19)	0	4
R3	1.1	0	12	**	11(72 \pm 13)	0	0
R4	3.2	0	12	**	12(53 \pm 7)	0	0
R5	10.5	0	9	**	8(38 \pm 6)	0	0

- *1) Fractures apparent at doses R3 through R5.
 2) Blood dysplasias apparent at doses R2 through R5.
 3) Tooth loss apparent at doses R1.7 through R5.
 4) Gingivitis apparent at R5 dose level only.

**No dogs currently living.

2.5. Skeletal toxicity in adult beagles (5 years old at injection)

It remains to be seen whether the induction of bone sarcoma can occur as readily in old individuals. The longer residence time on bone surfaces and the further accumulation of ^{239}Pu on those same surfaces due to the ^{239}Pu recycled from the liver should favour increased induction. On the other hand, the number of sensitive cells near bone surface is markedly reduced with age; thus, one can postulate a decrease in sensitivity. A determining factor will probably be the short remaining lifespan which will obviously limit the appearance of tumours at low doses for the beagle (8 post-injection years for adults versus 11.5 years for young adults).

To shed some light on whether adults are more or less sensitive, we have just instituted a five-year-old adult beagle toxicity study of ^{239}Pu and ^{226}Ra utilizing rib and liver biopsies (Table VII). These biopsies are to be performed at intervals up to 5 years post-injection. Due to the difficulties in obtaining 5-year-old beagles, the injection of these dogs will not be completed until 1979.

2.6. Skeletal toxicity in rapidly growing beagles (3 months old at injection)

The shorter residence time of ^{239}Pu on bone surfaces from rapid bone modelling and remodelling in growing beagles should favour a reduced sensitivity. Even though there is a larger number of bone cells near bone

Table VII. Experimental Design of Pu-239/Ra-226/Biopsy/Toxicity/Adult Beagle Study*

Code	Dose $\mu\text{Ci/kg}$	Male	Female	Total
P3	0.90	3	3	6
P2	0.095	3	3	6
P1.7	0.048	6	6	12
P1	0.015	6	6	12
R5	10.5	6	6	12
R4	3.1	6	6	12
R3	1.1	6	6	12

*Initiated in 1976 and injection completed by 1979.

Table VIII. Current Status of Pu-239/Toxicity/Pilot/Beagle Puppies Study

Code	$\mu\text{Ci/kg}$	Alive	Dead	Range Post- injection months (living beagles)	No. with osteosarcoma
P0	0	5	0	34-36	0
P1	0.015	1	0	104	0
P3	0.300	3	1	34	1(93)**
P5	2.700	6*	6	34-36	6(39 \pm 2)**

* Three dogs bearing bone tumors.

** Latent period in months \pm S.D.

Young adult P3 latent period was 55 \pm 7 months.

surfaces with higher proliferative activity in growing bones, the shorter residence time of bone surface ^{239}Pu will reduce the actual number of sensitive cells at risk.

Currently our pilot puppy studies are approaching a 100% incidence of osteosarcoma after 39 \pm 2 months post-injection of 2.7 $\mu\text{Ci/kg}$ as compared to about 80% incidence at 45 \pm 10 months in young adults (Table VIII). One bone sarcoma was observed in a dog with 0.3 $\mu\text{Ci } ^{239}\text{Pu/kg}$ at 93 months post-injection as compared to an average osteosarcoma appearance time of 55 \pm 7 months in young adults injected with the same dose level. The latent period of this one bone tumour suggests a lower sensitivity in rapidly growing animals.

In order to study better whether rapidly growing dogs are more or less sensitive to bone tumour induction, we have started a full-scale study of beagles injected at three months of age with ^{239}Pu and ^{226}Ra using 12 dogs per dose level with half of them subjected to rib and liver biopsies (Table IX).

Table IX. Experimental Design of Pu-239/Ra-226/Toxicity/Beagle Puppies Study

Code	Dose ($\mu\text{Ci/kg}$)	No. of Beagles	Code	Dose $\mu\text{Ci/kg}$	No. of Beagles
P0	0	12*	R0	0	12*
P0.5	0.005	12*	R0.5	0.02	12*
P1	0.015	12*	R1	0.06	12*
P2	0.095	12*	R2	0.35	12*
P3	0.3	12*	R3	1.1	12*

* 6 biopsied dogs per dose level. The biopsy times are at 0.08, 1.15, 0.34, 0.71, 1.5, 1.9, 2.4, 3.1, 3.9 and 5.0 yr.

2.7. Skeletal toxicity as a function of age (summary)

In due time, we should know whether the past studies employing young adults are applicable to older and younger members of the population. Currently, there is insufficient information to make an estimate. It remains to be seen whether our postulated decreased sensitivity in rapidly growing beagles and increased sensitivity in adult beagles for radionuclide-induced bone tumour will stand up.

2.8. Skeletal toxicity in St. Bernards

Since the St. Bernard and the beagle are at the opposite ends of the sensitivity spectrum for naturally occurring bone cancer [29], these two breeds appear ideal for evaluation of the sensitivity factors — an important consideration in establishing risk estimates for the entire human population. Early results from the pilot St. Bernard study show a direct relationship between spontaneous and radiation-induced bone tumour sensitivity; therefore, the latent period for osteosarcoma induced in this giant breed by ^{239}Pu is approximately one-half or less than that of comparable dose levels in the beagle (Table X) (Taylor, G.N., personal communication).

2.9. Skeletal toxicity of polymeric ^{239}Pu in beagles

A pilot study on a single injection of polymeric ^{239}Pu in young adult beagles (17 months old) has yielded some significant observations. The polymeric ^{239}Pu [32, 33] is initially deposited in the liver. There is about 70% of the retained ^{239}Pu in the liver and less than 3% in the bones at day 14. As the liver released its ^{239}Pu , some of it was distributed to the bone 1100 days post-injection, the retention in the liver was about 30% and the skeleton was about 25% (Table XI). Autoradiographically, the ^{239}Pu at 1100 days was seen to be principally on bone surfaces, which suggests that the bulk of the ^{239}Pu arrived at the bone surface of a mature skeleton. Two out of two dogs succumbed to bone sarcoma at 39 ± 2 months compared to a

Table X. Current Status of Pu-239/Toxicity/Pilot/Young Adult St. Bernard Study

Code	Dose ($\mu\text{Ci/kg}$)	Alive	Dead	Post-injection months (alive)	No. with osteosarcoma
0.1E	0.0006	3	0	0-18	0
0.2E	0.0018	3	0	18-19	0
0.5E	0.005	3	0	33	0
1.0E	0.015	3	0	33-34	0
2.0E	0.095	0	3	**	3(49 \pm 8) *
3.0E	0.3	0	3	**	3(30 \pm 4) *
4.0E	0.9	1	0	(32)	0

*latent period in months \pm S.D.

P3 young adult beagles latent period of 55 \pm 7.

P2 young adult beagles latent period of 87 \pm 17.

**no dogs currently living.

Table XI. Skeletal Retention and Toxicity of Polymeric (P) and Monomeric (M) ^{239}Pu *

Form of Pu	No. Dogs	Days Post-Injection	% Retention of injected ^{239}Pu skeleton	liver	Cause of Death
Pu-(M)	1	14	48.9	32.5	Sacrifice
Pu-(P)	1	14	2.2**	68.2	Sacrifice
Pu-(P)	1	1184	23.4	31.9	Osteosarcoma
Pu-(P)	1	1148	25.5	29.6	Osteosarcoma
Pu-(M)	12	1319 \pm 183	41.2	16.1	Osteosarcoma

* Injected at 17 months with 0.9 $\mu\text{Ci/kg}$.

**Principally in marrow.

latent period of 45 \pm 7 months in monomeric ^{239}Pu injected at the same dose level (0.9 $\mu\text{Ci } ^{239}\text{Pu/kg}$) [34]. If these polymeric dogs mimic the adult skeleton toxicity model, one can postulate similar arguments for the 5-year-old beagle. Needless to say, this is a very stimulating model and more studies are needed, especially at lower injected dose levels.

3. FUTURE RESEARCH

This is an exciting period for the Utah programme. Our chief interest will continue to be plutonium for "this is the element of the nuclear age" [35]. The emphasis will be on low-dose effects as a function of age. Currently we have the flexibility in our colony to carry out short-term experiments to improve the mathematical models of the temporal dose pattern and cell kinetic activity prior to tumour formation. Our policy of bone and liver biopsies in chronic toxicity dogs will allow us to obtain early retention and microdosimetric data as well as mechanism of binding data to predict toxicity while we patiently await possible late effects to arise.

Recent developments in improved bone and bone marrow histology [35-38], quantitative morphology, neutron-induced ^{239}Pu autoradiography [39, 40], cell kinetics studies of liver and bone, as well as metabolic studies using ^{237}Pu [41], will enable us to better understand the biological effects of low doses of internally deposited radionuclides.

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DISCUSSION

K. NEUMEISTER: Is it possible to estimate the cancer-inducing doses in the skeleton in rads or rems in your experiments? In my opinion this would be of general interest in connection with cancer induction in bones after low doses, especially for purposes of comparison with other radionuclides.

W.S.S. JEE: This has been done by Dr. C.W. Mays and co-workers using average skeletal dose. He also deals with the RBE of Pu/Ra in paper IAEA-SM-202/806 in these Proceedings.

Helen WOODARD: Dr. Jee has demonstrated how plutonium deposits on the bone surfaces may be buried by bone growth in young dogs, with the result that the osteoprogenitor cells are no longer exposed. In the dog, as in man, there is a clearly defined long adult period during which there is no net growth and little remodelling, and hence little translocation of plutonium from bone surfaces. In the mouse there is no well defined adult period but there is a net gain of both tibia length and bone mineral up to 15 months of age. I believe the rat is similar to the mouse in this respect. Hence a pulse label of plutonium is likely to be separated very promptly from the osteoprogenitor cells even in older mice. Results will probably be very different from those in the dog.

W.S.S. JEE: I fully agree with Dr. Woodard. For those who cannot work with adult animals, there still is significant information to be obtained from rat and mice studies. There is a wealth of data on the distribution of progenitor cells in the mouse as a function of age, which should be correlated with the effect of age on the distribution of ^{239}Pu on bone surface. This work is needed in order to improve our understanding of the factors affecting bone sarcoma sensitivity.

Miriam FINKEL (Co-chairman): Is the location of bone tumours in the dogs treated at three months of age the same as that in dogs treated as young adults?

W.S.S. JEE: The locations are not quite the same. The tumours of the three-months-old animals are located principally in the vertebral column, while those of the young adults are located in the vertebral column and the long bones.

Miriam FINKEL: Are there multiple bone tumours in the very young dogs, as there are in the young adults?

W.S.S. JEE: The search for microscopic tumours in the three-months-old animals is in progress. There are no radiographically detectable multiple bone tumours.

The distribution of osteosarcoma in beagles injected at three months of age is as follows:

M81P5Y	proximal humerus
F82P5Y	L-3 vertebra
F84P5Y	proximal tibia

	F85P5Y	frontal bone
	F87P5Y	L-5 vertebra
	F88P5Y	distal femur
	M90P5Y	T-12 vertebra
Living:	M86P5Y	proximal femur
	M89P5Y	distal tibia
	M92P5Y	proximal femur
	T50P3	C-5 vertebra and mandible
	T51P5	distal humerus

There is one dog injected with $0.3 \mu\text{Ci/kg}$ with a multiple tumour at the fifth cervical and mandible. The distribution of tumours was quite similar to that of the young adults.

M. DELPLA: In Table VI you mention ten cases of eye melanoma in dogs contaminated by ^{226}Ra . To my knowledge, eye melanoma has never been observed in persons contaminated by this radionuclide. Is this due to chance (small human samples) or to difference between species?

W.S.S. JEE: This is because of the species difference. I suggest you look up the definitive studies of G.N. Taylor (University of Utah) and of Fisher (University of California at Davis).

M. GOLDMAN: I may here add that, regardless of age, the dog eye is anatomically different from the human. In the dog, the form of the tapetum lucidum apparently promotes the concentration of large quantities of ^{226}Ra in and about the melanocytes. Doses associated with melanoma of the eye are of the order of 50 rad/d .

GREFFE CHEZ LA SOURIS ATHYMIQUE DE TISSU PULMONAIRE DE RAT CONTAMINE PAR L'OXYDE DE PLUTONIUM

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Abstract-Résumé

GRAFTS OF PULMONARY TISSUE OF RATS CONTAMINATED WITH PLUTONIUM OXIDE IN ATHYMIC MICE.

Pulmonary fragments from control rats (Wistar) of varying age and contaminated with PuO_2 were grafted in an immunodeficient medium onto mice or histocompatible rats. In both cases the authors observed lesions presenting the histological characteristics of pulmonary cancer in rats; proliferation, however, remained discrete. After grafting on the athymic mice, the lungs of the control animals exhibited a percentage of tumoral-type differentiations which increased with age. In the contaminated rats, for an initial pulmonary burden of 10 nCi and cumulative doses of between 6×10^8 and 38×10^9 α/g of lung, the grafts showed a clear-cut increase in lesions of this type. Their frequency increased with the total dose delivered. Conversely, if the dose delivered during a three-month period varied from 2×10^8 to 50×10^9 α/g of lung, the maximum proliferation was observed at the lowest doses. Furthermore, the histological nature of the proliferations varied with the dose delivered. This transplantation technique would appear to show the existence of lesions which are not yet overt and which are a consequence either of ageing or of α -irradiation. The authors discuss the meaning of these lesions and the immunological component of the phenomenon.

GREFFE CHEZ LA SOURIS ATHYMIQUE DE TISSU PULMONAIRE DE RAT CONTAMINE PAR L'OXYDE DE PLUTONIUM.

Des fragments pulmonaires provenant de rats Wistar témoins d'âge variable contaminés par l'oxyde de plutonium ont été greffés en milieu immunodéficient soit chez la souris, soit chez le rat histocompatible. Dans les deux cas il apparaît des lésions ayant les caractères histologiques des cancers pulmonaires de rat, mais dont la prolifération demeure discrète. Les poumons d'animaux témoins présentent, après passage sur la souris athymique, un pourcentage de différenciations de type tumoral qui augmente avec le vieillissement. Chez les rats contaminés, pour une charge pulmonaire initiale de 10 nCi et des doses cumulées comprises entre $6 \cdot 10^8$ et $38 \cdot 10^9$ α/g de poumons, les greffons montrent une augmentation nette des lésions de ce type. Leur fréquence croît avec la dose totale délivrée. Par contre, si la dose délivrée pendant trois mois varie de $2 \cdot 10^8$ à $50 \cdot 10^9$ α/g de poumons, le maximum de proliférations est observé aux doses les plus faibles. Par ailleurs, la nature histologique des proliférations varie avec la dose délivrée. Cette technique de transplantation paraît mettre en évidence des lésions encore inapparentes et consécutives soit au vieillissement, soit à l'irradiation α . La signification de ces lésions et la composante immunologique du phénomène sont discutées.

INTRODUCTION

La contamination pulmonaire par les radioéléments émetteurs de particules α provoque chez le rat des lésions variables en fonction de la dose délivrée [1]. Les premiers cancers sont observés 250 jours après l'inhalation pour des doses supérieures à 10^2 «rad équivalent curium», c'est-à-dire $6 \cdot 10^9$ α/g de poumons dans le cas de PuO_2 [2]. Il a été également démontré [3] que des doses inférieures à 10^2 rad provoquent chez le rat un

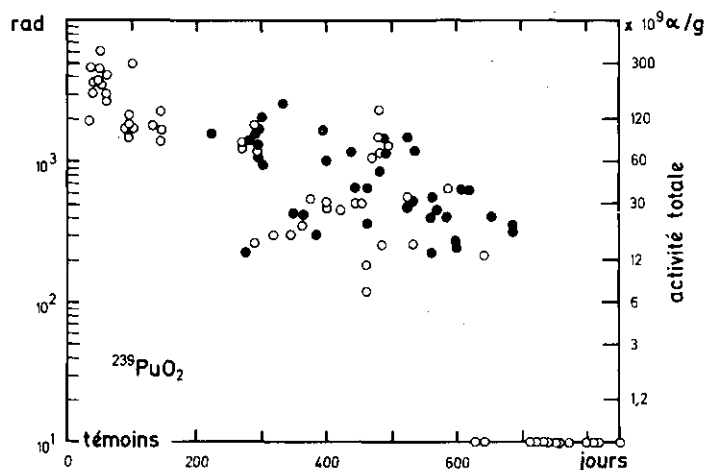


FIG.1. Relations entre la dose, la survie et la fréquence des tumeurs pulmonaires après inhalation de $^{239}\text{PuO}_2$:
 ○ sans cancer; ● avec cancer.

raccourcissement de la durée de vie indépendant de la mortalité due aux cancers. En outre, les émetteurs α partiellement solubles provoquent une augmentation très nette des tumeurs extra-pulmonaires bien que la dose délivrée aux différents tissus autres que le poumon soit très faible. Au cours de cette étude nous décrirons l'évolution histologique d'explants pulmonaires greffés chez des animaux histocompatibles. Ces fragments de tissu pulmonaire sont prélevés chez des rats à différents intervalles de temps après l'inhalation de $^{239}\text{PuO}_2$, mais avant l'apparition des cancers pulmonaires.

Le but de ce travail est d'étudier le tissu pulmonaire placé dans un environnement, dépourvu d'immunité à médiation cellulaire, dans lequel il peut survivre et révéler d'éventuelles modifications apportées par l'irradiation ou par le vieillissement tissulaire.

MATERIEL ET METHODES

Animaux

Les donneurs sont des rats soit de souche Wistar «outbred», soit de souche Wistar AG «inbred». Ils sont élevés en milieu conventionnel et ont une durée de vie moyenne de 850 jours. Les conditions d'empoussiérage des rats par l'oxyde de plutonium ($^{239}\text{PuO}_2$) ont été précédemment décrites [4]. Les particules utilisées ont un diamètre réel moyen de $0,6 \mu\text{m}$ et sont produites par grillage du peroxyde à 1000°C . Les relations entre la dose, la survie et la fréquence des tumeurs sont portées sur la figure 1.

Les receveurs sont des souris athymiques¹ obtenues par croisement de mâles BALB/C homozygotes pour le gène nu et de femelles NCS hétérozygotes pour ce même gène [5]. Elles sont conservées dans les conditions conventionnelles à 27°C ; leur durée de vie moyenne est de 80 jours.

¹ Les géniteurs nous ont été aimablement fournis par le Laboratoire de biologie moléculaire de l'Institut Pasteur, à Paris.

Greffes

Pour chaque donneur trois souris reçoivent sous la peau cinq explants de tissu pulmonaire. Les explants (2 mm^3) sont pris au hasard dans les différents lobes pulmonaires. Une souris est systématiquement sacrifiée après 21 jours, les deux autres lorsqu'elles sont moribondes. Lorsque l'inhalation est effectuée sur des rats Wistar AG consanguins, le devenir des explants pulmonaires est comparé sur des souris athymiques, des rats thymectomisés à la naissance [6] et des rats témoins.

Analyse histologique des résultats

Pour chaque prélèvement une partie du tissu pulmonaire est greffée et l'autre immédiatement fixée par le mélange glutaraldéhyde-paraformaldéhyde. Après passage chez les receveurs, les greffons sont fixés selon les mêmes modalités puis inclus dans l'araldite. L'analyse histologique est faite au microscope optique pour les coupes semi-fines et au microscope électronique dans les cas litigieux. Par ailleurs, les poumons des donneurs sont fixés au Bouin Hollande et traités en coupes histologiques sériées.

RESULTATS

Evolution des greffons témoins

L'étude de l'évolution du greffon de tissu pulmonaire en fonction de l'âge du donneur a été effectuée sur 34 rats témoins. Les animaux, divisés en six groupes, ont été sacrifiés à 45 jours, 3, 6, 12, 14 et 24 mois. Les greffons ont été observés jusqu'à 70 jours après leur implantation. Le tissu est totalement vascularisé après 4 à 5 jours. Chez les animaux jeunes, tous les explants conservent une architecture normale (fig.2). Lorsque l'âge des donneurs augmente les greffons prennent un aspect qui permet de différencier plusieurs groupes:

1°) L'architecture générale du tissu est conservée et la cellularité est peu modifiée.

2°) L'architecture générale du tissu est conservée mais il apparaît une hyperplasie considérable des pneumocytes type II, hypersécrétants, groupés en pseudo-acini dont la lumière est occupée par un amas de corps lamellaires. Cette modification est fréquemment accompagnée d'une prolifération de fibroblastes. Dans notre nomenclature cette lésion est qualifiée de «douteuse».

3°) Le tissu est remanié par la fibrose malgré une vascularisation correcte. Deux évolutions sont possibles: l'une conduit à une hypercellularité due à la multiplication des fibroblastes, des cellules endothéliales et de pneumocytes II atrophiques, l'autre se traduit par un dépôt plus intense de collagène accompagné d'une disparition des pneumocytes II tandis que demeure au moins pendant un mois le recouvrement de pneumocytes I.

4°) L'architecture n'est pas conservée, des formations nodulaires ou papillo-végétantes apparaissent. Elles sont composées de cellules indifférenciées, de cellules proches des cellules de Clara, de pneumocytes II pauvres en corps lamellaires. Cette réaction du tissu est considérée comme «positive» dans notre description (fig.3, 4 et 5).

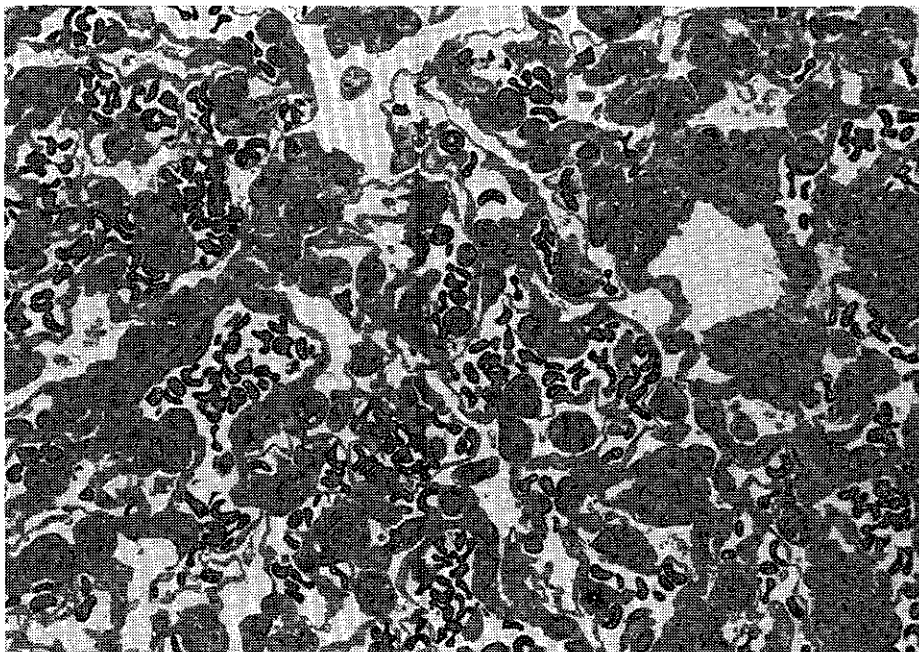


FIG.2. Coupe semi-fine — coloration Azur II. Aspect après 21 jours d'une greffe pulmonaire du rat jeune (45 j).

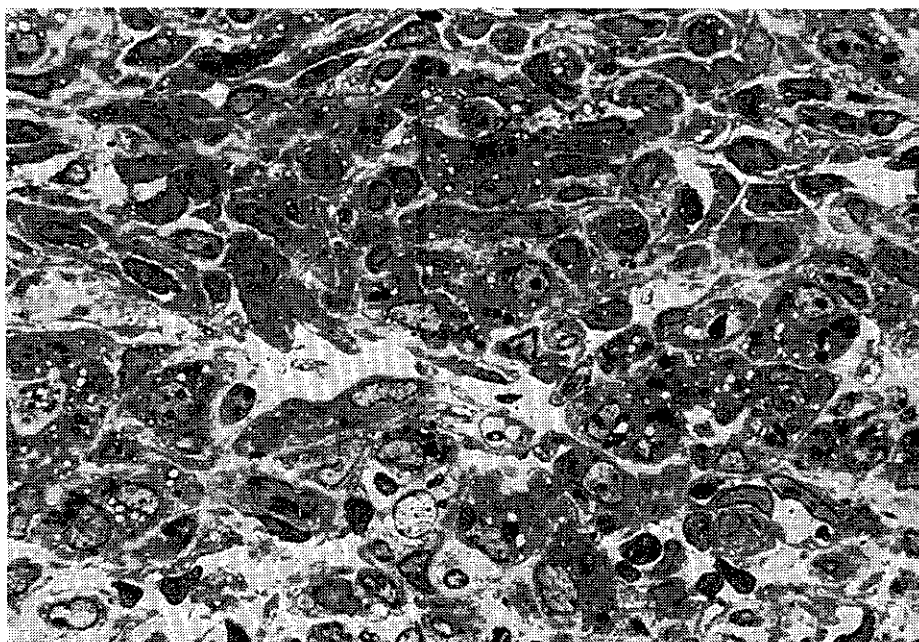


FIG.3. Coupe semi-fine — coloration Azur II. Disparition de l'architecture pulmonaire par prolifération de pneumocytes II et de cellules peu différenciées d'origine bronchiolaire au 21^e jour après la greffe de tissu pulmonaire d'un rat de deux ans.

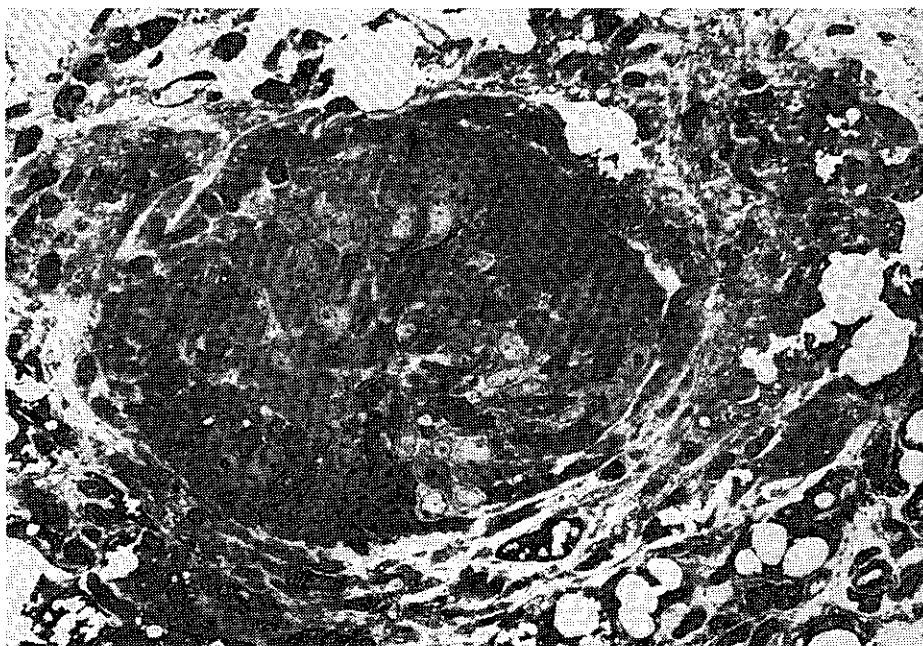


FIG.4. Coupe semi-fine — coloration Azur II. Greffon: rat témoin de deux ans — Prolifération nodulaire après 21 jours.

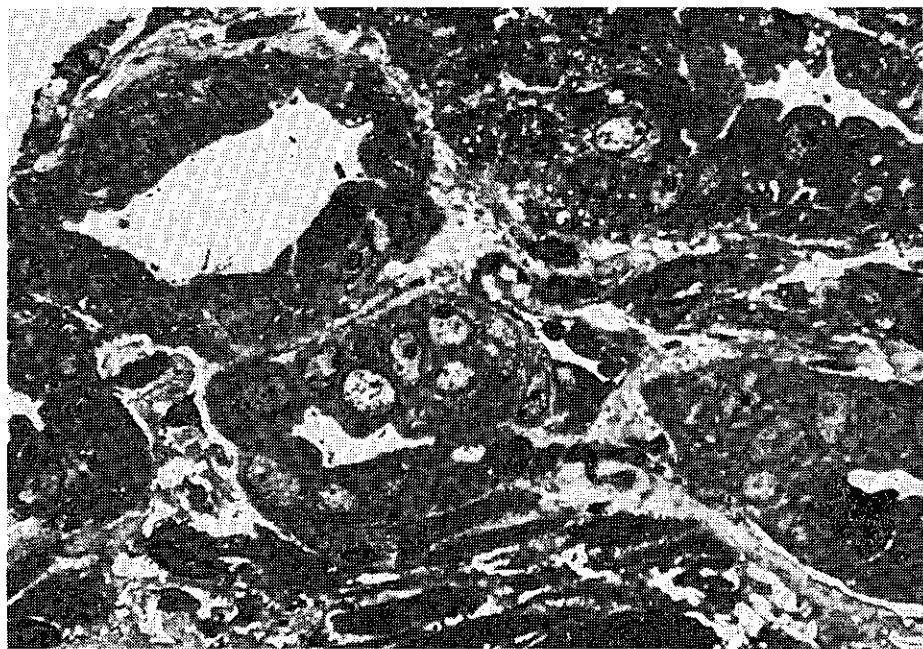


FIG.5. Coupe semi-fine — coloration Azur II. Greffon: rat témoin de deux ans — Prolifération papillo-végétante après 21 jours.

TABLEAU I. POURCENTAGE DE TRANSFORMATIONS EN FONCTION DE L'AGE DES TEMOINS

Donneurs: Wistar témoins		Receveurs: souris athymiques				
Age au moment du prélèvement	% de transformations positives			% de transformations douteuses		
	A*	B**	C***	A*	B**	C***
45 jours	0	0	0	0	0	0
3 mois	3	4	5	10	11	15
6 mois	5	13	18	9	25	30
12 mois	8	19	21	11	27	29
14 mois	10	19	22	8	16	18
24 mois	8	23	27	8	23	27

A* % par rapport au nombre total de greffons

B** % par rapport aux greffons vascularisés

C*** % par rapport aux greffons vascularisés et non fibrosés.

TABLEAU II. POURCENTAGE DE TRANSFORMATIONS EN FONCTION DU TEMPS APRES L'INHALATION

Donneurs: Wistar			Receveurs: souris athymiques					
Dose délivrée α	Survie après inhalation (jours)	Age au prélèvement (jours)	% transformations positives			% transformations douteuses		
			A*	B**	C***	A*	B**	C***
$6 \cdot 10^8$	30	120	12	20	27	6	11	14
$4 \cdot 10^9$	240	230	15	27	30	8	16	18
$16 \cdot 10^9$ (7-38 $\cdot 10^9$)	300 à 440	360 à 500	36	49	53			

A* B** C***: voir tableau I.

Les résultats obtenus sont reportés sur le tableau I. Par rapport au nombre de greffons vascularisés, on observe une nette augmentation des proliférations avec l'âge: 23% chez les animaux âgés de deux années. Ce pourcentage exprimé en fonction des greffons non fibrosés présente une évolution identique. Si l'on rapporte les résultats au nombre total de greffons, l'augmentation en fonction de l'âge est masquée par l'évolution de la prise des greffons (voisine de 80% pour les poumons d'animaux jeunes et inférieure à 40% chez les animaux âgés de deux ans). L'évaluation par rapport au nombre de greffons vascularisés nous semble plus judicieuse car elle met en relief la réalité du phénomène au niveau des cellules demeurées fertiles.

Evolution en fonction du temps des greffons irradiés

A cette évolution du comportement des greffons due au vieillissement peut être comparée l'évolution du comportement des greffons irradiés. A cette fin, 22 Wistar «outbred» ayant inhalé environ 10 nCi de PuO_2 sont répartis en trois groupes et sacrifiés à 30, 240 et 300-440 jours après l'inhalation. Le tableau II montre que l'augmentation du nombre des greffons positifs est évidente à tous les âges par rapport aux témoins. Elle se manifeste très précocement.

Du point de vue histologique, les transformations positives chez les irradiés comportent trois types de proliférations: la prolifération bronchiolo-alvéolaire précédemment observée chez les témoins; une prolifération de cellules endothéliales et de capillaires incomplets conduisant à un véritable angiome détruisant l'architecture du greffon (fig.6) (cette lésion est qualifiée

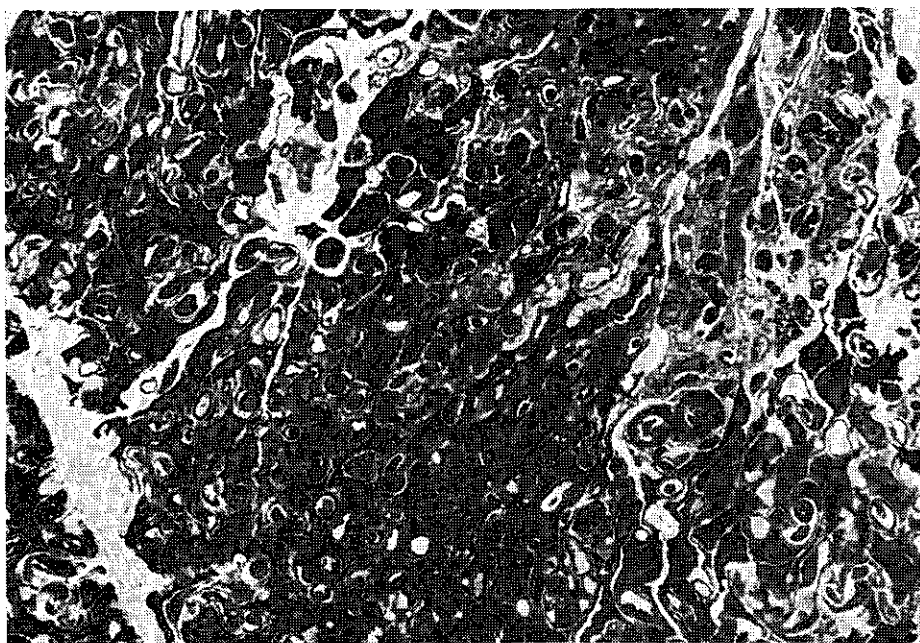


FIG.6. Coupe semi-fine — coloration Azur II. Greffe de poumon irradié à $2 \cdot 10^9$ α/g de poumons après 120 jours — Angiomatose au 21^e jour.

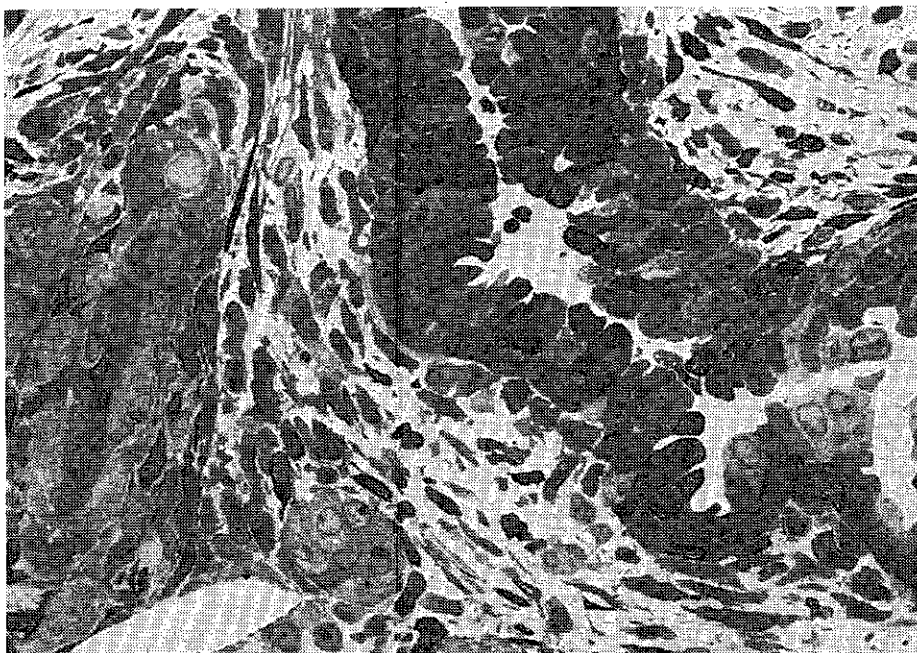


FIG.7. Coupe semi-fine — coloration Azur II. Greffe de poumon irradié à $7 \cdot 10^9$ α/g de poumons après 400 jours — Prolifération épidermoïde après 10 jours.

d'angiomatose; elle a été observée une fois dans un tissu témoin); enfin, des proliférations de type épidermoïde sont mises en évidence, toujours dans les prélèvements comportant un fragment bronchique (fig.7). Ces deux derniers types cellulaires n'apparaissent que dans le lot d'animaux dont la survie est la plus longue. Les proliférations bronchiolo-alvéolaires ne représentent plus dans ce dernier groupe qu'environ 50% des proliférations contre 100% dans les groupes à doses plus faibles; 40% des lésions sont de type angiomatose et 10% de type épidermoïde.

Relation dose-effet

Sur trois lots de Wistar AG la greffe a été réalisée 120 jours après l'inhalation, la charge pulmonaire initiale étant respectivement de 1, 10 et 100 nCi. Les résultats (tableau III) indiquent qu'un très fort pourcentage (70%) de proliférations est obtenu à dose faible. Lorsque la dose délivrée augmente, on observe un véritable effet radiothérapique puisque seulement 10% des greffons présentent une positivité pour la plus forte dose. On observe également qu'en fonction de la dose délivrée les types cellulaires prolifératifs sont différents (fig.8).

Cette expérience a été complétée par une étude comparée de l'évolution des greffons sur des rats consanguins thymectomisés à la naissance ou non.

Les résultats obtenus (tableau IV) mettent en évidence une prolifération plus discrète chez le rat normal que chez le rat thymectomisé. L'allure générale du phénomène précédemment décrit chez la souris athymique est conservée; toutefois, les valeurs obtenues ne montrent pas de manière aussi nette l'effet stérilisant des fortes doses. Il est cependant à noter que le nombre de rats consacrés à cette étude est faible (50% des animaux étant conservés en survie pour une étude à long terme); de ce fait les valeurs obtenues n'ont pas la même signification statistique que chez les souris athymiques.

Dans quelques cas, les greffons ont subi un second passage chez des souris athymiques. Les lésions positives conservent dans ce cas leurs caractères histologiques sans pour cela prendre un aspect envahissant typique des tumeurs établies.

DISCUSSION

Nous avons transplanté avec succès et sans perte de malignité chez la souris nude trois carcinomes épidermoïdes, deux carcinomes bronchiolo-alvéolaires et un hémangio-sarcome. Deux cancers pulmonaires, épidermoïde et fibro-sarcome dérivé d'une stroma-réaction, sont ainsi maintenus depuis plus d'un an par passage sur la souris athymique. Ces résultats sont en accord avec les observations publiées [7 - 9].

L'apparition de proliférations cellulaires chez des explants provenant d'animaux témoins ou irradiés, sans lésion histologique visible, est une observation nouvelle; toutefois, la nature de ces proliférations cellulaires doit faire l'objet d'une discussion particulière.

Rappelons que chaque fragment de poumon prélevé est pour une part analysé histologiquement, pour une part greffé. Les poumons sont de même totalement étudiés lobe par lobe. Quelques fragments présentant de l'adénomatose ou de la métaplasie épidermoïde ont également été greffés par ailleurs et sont toujours demeurés stériles.

Compte tenu de l'aspect histologique normal avant la greffe et de la fréquence observée chez les greffons, la possibilité d'une greffe de lésion pré-établie semble exclue. Il s'agit donc de proliférations spécifiques apparaissant avec une forte incidence chez les animaux privés d'immunité à médiation cellulaire.

Ces proliférations présentent des analogies histologiques évidentes avec les tumeurs pulmonaires produites chez le rat par irradiation pendant un temps plus important. Les proliférations de cellules bronchiolaires indifférenciées et de pneumocytes II rappellent les formations de tumeurs bronchiolo-alvéolaires [10]. Elles sont parfois parfaitement organisées en cordons compacts et infiltrants. Des formes d'adéno-carcinome ont été parfois observées. Les lésions d'angiomatose présentent des coalescences de vaisseaux sanguins composés de cellules endothéliales atypiques, faiblement jointives, reposant sur une membrane basale. Ouverts en de nombreux points dans l'espace conjonctif ils offrent une analogie certaine avec les angio-sarcomes. Enfin, les proliférations épidermoïdes, les moins fréquentes, sont histologiquement comparables aux cancers épidermoïdes que nous avons observés chez le rat.

La comparaison avec les tumeurs qui apparaissent normalement chez le rat après inhalation de PuO_2 doit se limiter à cette analogie histologique.

TABLEAU III. POURCENTAGE DE TRANSFORMATIONS EN FONCTION DE LA DOSE DELIVREE PENDANT UNE DUREE CONSTANCE

Donneurs: Wistar AG			Receveurs: souris athymiques					
Survie après inhalation (jours)	Age au moment de la greffe (jours)	Dose délivrée (α/g)	% transformations positives			% transformations douteuses		
			A*	B**	C***	A*	B**	C***
120	210	$2 \cdot 10^8$	40	70	70	7	12	12
120	210	$2 \cdot 10^9$	11	16	20	7	10	13
120	210	$50 \cdot 10^9$	5	8	10	7	12	14

A* B** C***: voir tableau I.

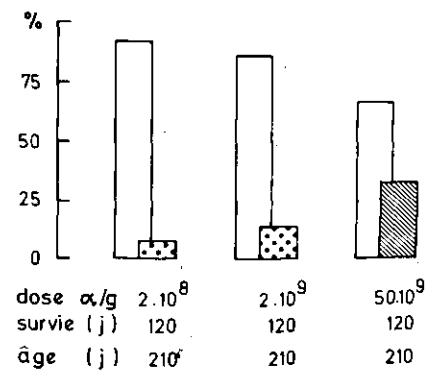



FIG.8. Type histologique des proliférations positives, en fonction de la dose délivrée:  bronchiolo-alvéolaire;  angiomatose;  épidermoïde.

TABLEAU IV. POURCENTAGE DE PROLIFERATIONS CHEZ DIFFERENTS RECEVEURS

Dose délivrée α	Pourcentage de proliférations					
	Positives			Douteuses		
	Souris athymique	Rat normal	Rat thymectomisé	Souris athymique	Rat normal	Rat thymectomisé
$2 \cdot 10^8$	70	21	45	12	0	0
$2 \cdot 10^9$	20	10	22	13	10	11
$50 \cdot 10^9$	10	10	40	14	20	50

En effet, la multiplication cellulaire obtenue chez les greffons n'a aucune commune mesure avec la croissance rapide des greffes tumorales. La seule expérience utilisant des rats syngéniques n'a montré que l'apparition de ces organisations compactes de cellules indifférenciées à caractère prolifératif discret. Au cours des transplantations successives, les transformations observées sont maintenues sans acquérir de caractère prolifératif plus important. Un certain nombre de ces lésions transplantées sur le rat sont encore en observation; après trois mois aucune prolifération n'a été observée. Toutefois la visualisation d'un dommage encore latent chez les donneurs et apparaissant avec une plus forte incidence en milieu immunologiquement déprimé ne peut être contestée. Plusieurs problèmes sont soulevés par cette observation. Pour une très grande part, les lésions observées sont identiques et simplement plus fréquentes chez les animaux irradiés que chez les témoins. L'irradiation apparaîtrait donc comme un simple facteur d'accélération d'un phénomène étroitement lié au vieillissement.

Malgré le caractère théoriquement très hétérogène de la distribution de dose, le tissu pulmonaire présente des lésions réparties de façon très homogène. Il faut donc admettre, soit que le mouvement des particules dans les macrophages alvéolaires assure une répartition homogène des dommages, soit qu'il existe une information globale au niveau de l'organe à partir d'une irradiation localisée. Si l'on admet que ces lésions sont de même nature que les cancers pulmonaires observés chez le rat, ou peuvent participer à leur genèse, il faut également admettre que la surveillance immunitaire ayant pour origine les lymphocytes T ne permettrait que le développement de lésions initiales. L'acquisition de caractère néoplasique résulterait de la conjonction d'autres mécanismes locaux ou généraux, ce que semblerait confirmer le fait que les cancers spontanés, chez la souris athymique, sont pratiquement inexistants [11,12].

REMERCIEMENTS

Ce travail a été effectué avec l'assistance technique de Mesdames Beauvallet, L'Hullier, Vanelle et de Monsieur Discour.

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DISCUSSION

Miriam FINKEL (Co-chairman): When the lung tissue was transplanted, was any plutonium transferred with it?

D. NOLIBE: The presence of plutonium in the grafts is checked by autoradiography. In some cases, particles were indeed present but we did not observe any direct relationship between the location of particles and proliferation, whereas numerous proliferations occurred in grafts free from PuO₂ particles.

Miriam FINKEL: What would you estimate the earliest time to be for the neoplastic change to occur in the lung tissue?

D. NOLIBE: In the present experiment, a very significant increase in relation to the controls was observed when the grafting was carried out 30 days after inhalation, the duration of the graft being then 21 days. But we do not yet know whether this time is the minimum or whether it can be reduced further.

INDUCTION DE CANCERS CHEZ LE RAT APRES INHALATION DE RADIOELEMENTS EMETTEURS ALPHA

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Abstract-Résumé

INDUCTION OF CANCERS IN THE RAT AFTER INHALATION OF ALPHA-EMITTING RADIONUCLIDES.

Experiments have been conducted for several years on the toxic action of inhaled alpha-emitters on laboratory rats. The inhaled radionuclides were ^{239}Pu , ^{238}Pu and ^{241}Am in oxide and nitrate form. The initial alveolar activities varied to give total activities ranging from 1 to 200 thousand million alpha-particles per gram of lung. Variations, depending on the physico-chemical form of the nuclides, in the space and time distributions of the dose are observed. The influence of the two parameters on life span reduction, latency time and frequency of cancer occurrence, initial location of tumours and their histological types were studied. A theoretical model relating the cancerogenous effect to the dose is compared with experimental data.

INDUCTION DE CANCERS CHEZ LE RAT APRES INHALATION DE RADIOELEMENTS EMETTEURS ALPHA.

Une expérimentation a été entreprise depuis plusieurs années pour étudier l'action toxique d'émetteurs alpha inhalés chez le rat de laboratoire. Les radioéléments inhalés ont été le plutonium-239, le plutonium-238 et l'américium-241 sous forme d'oxyde et de nitrate. Les activités initiales alvéolaires ont varié pour obtenir des activités totales allant de 1 à 200 milliards de particules alpha par gramme de poumon. Suivant la forme physico-chimique, on observe une variation des répartitions spatiales et temporelles de la dose. On a étudié l'influence des deux paramètres sur le raccourcissement de la durée de vie, le temps de latence et la fréquence d'apparition des cancers, la localisation initiale des tumeurs et leur type histologique. Un modèle théorique reliant la dose et l'effet cancérogène est comparé aux données expérimentales.

INTRODUCTION

Depuis plusieurs années, nous avons entrepris des recherches sur l'induction des cancers après inhalation de radioéléments émetteurs α . Actuellement, seules les expériences portant sur le plutonium-238 (oxyde et nitrate), le plutonium-239 (oxyde et nitrate) et l'américium-241 (oxyde et nitrate) sont terminées. Elles ont porté sur plus de 600 rats, et plus de deux cent cinquante cancers ont été observés.

Les doses cumulées délivrées dans ces expériences allaient, suivant les organes, de 10^7 à 10^{11} particules α par gramme de tissu frais. Exprimées en rad, les doses étaient comprises entre 1 et 10 000. Il n'est pas possible de traiter de façon isolée les doses les plus faibles car seule l'intensité des phénomènes et non leur nature dépend de la dose.

Les techniques de contamination et de mesure de la radioactivité [1] ont été décrites dans des publications antérieures ainsi que la méthode utilisée pour l'étude anatomo-pathologique des poumons [2].

*Association CEA/Euratom.

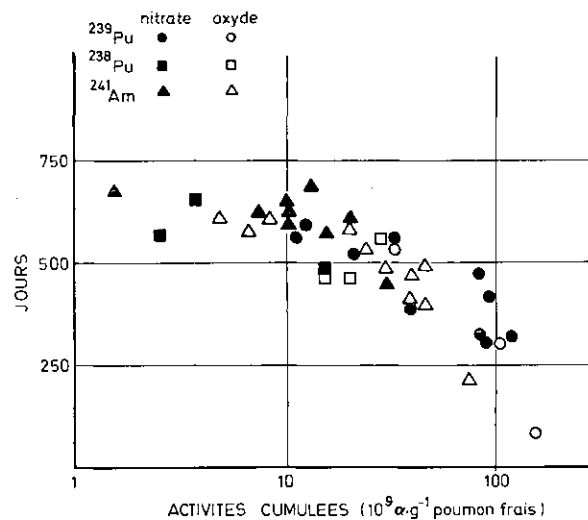


FIG. 1. Survie moyenne des groupes de rats ayant un cancer du poumon.

TABLEAU I. MORTALITE CUMULEE DES RATS AYANT UNE LIGNEE CELLULAIRE PULMONAIRE CANCEREUSE

Pu-239	f N %	Jours												
		< 250	< 300	< 350	< 400	< 450	< 500	< 550	< 600	< 650	< 700	< 750	< 800	< 850
		5 4,5	19 17	25 22	32 28	42 37	53 47	68 60	89 79	103 91	111 98	111 98	113 100	
Pu-238	f N %	1	1	1	2 4	4 13	8 22	10 49	14 58	15 74	15 85	15 86	15 87	15 88
Pu-238 Am-241	f N %	1 1	1 1	1 1	6 6	17 16,5	30 29	59 57	72 70	89 86	100 97	101 98	102 99	103 100

Pour les cancers des autres organes, leur présence a été décelée par une autopsie soigneuse et contrôlée par examen anatomo-pathologique.

Animaux en expérience et témoins ont été analysés suivant les mêmes méthodes.

1. TEMPS D'APPARITION DES TUMEURS

La première notion que l'on observe est que le temps de survie des animaux est d'autant plus long que la dose délivrée a été plus faible. Cette notion est vraie, que les animaux soient ou non porteurs d'un cancer. En règle générale, dans un groupe de rats ayant subi la même contamination, ce sont les animaux les plus âgés chez lesquels on observe les cancers. La figure 1 montre la relation entre la dose, exprimée en nombre total de particules α par gramme de tissu, et le temps moyen de survie des rats ayant un cancer du poumon.

Pour comparer le rôle du temps dans les cancers des différents organes, nous avons étudié les mortalités cumulées des animaux porteurs d'un cancer provenant de la même lignée cellulaire.

Poumons - Le tableau I montre la mortalité cumulée des rats ayant une lignée cellulaire pulmonaire cancéreuse pour deux conditions expérimentales: les composés de ^{239}Pu (oxyde et nitrate) lentement diffusibles et les composés à diffusion rapide (oxydes et nitrates de ^{238}Pu et ^{241}Am).

La même dose totale est donc délivrée de façon plus rapide dans le deuxième groupe et le débit de dose plus important entraîne une sclérose des tissus qui tue l'animal et empêche le développement des tumeurs.

La figure 2 met en évidence cette différence d'action des deux types de contaminants.

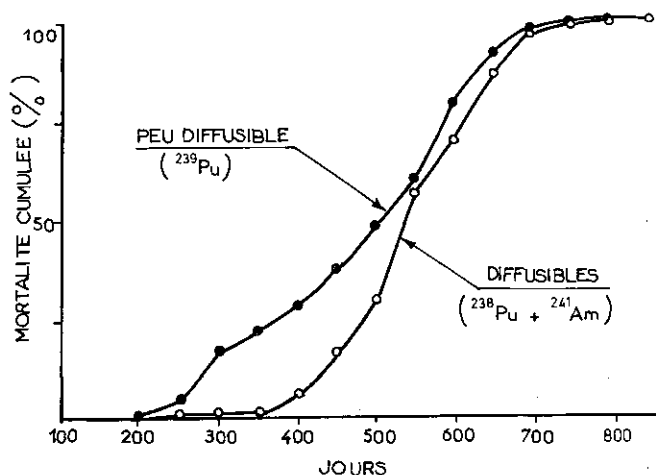


FIG. 2. Mortalité cumulée des rats ayant une lignée cellulaire pulmonaire cancéreuse.

TABLEAU II. MORTALITE CUMULEE DES RATS AYANT UN SARCOME (OS ET SANG)

		Jours								
		< 300	< 350	< 400	< 450	< 500	< 550	< 600	< 650	< 700
Os	f N	3	4	8	10	13	18	18	22	27
	%	11	14	29	36	46	64	64	79	96
Sang	f N			3	4	6	6	9	10	10
	%			30	40	60	60	90	100	100

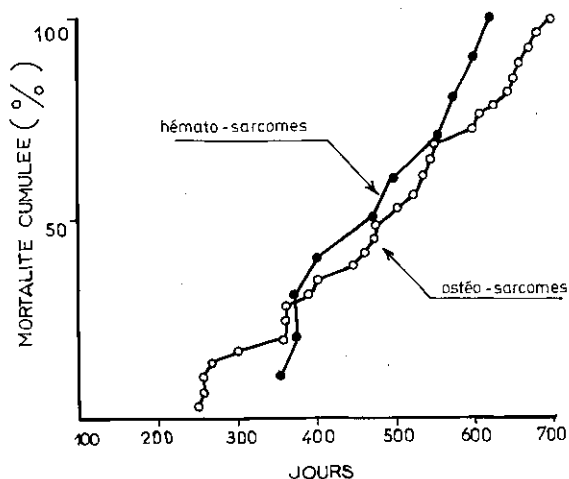


FIG. 3. Mortalité cumulée des rats ayant un ostéo-sarcome ou un hémato-sarcome en fonction du temps de survie.

Os et tissus «sanguins» — Les sarcomes de ces deux tissus ont une caractéristique commune qui est leur apparition précoce. Le tableau II et la figure 3 mettent bien ce fait en évidence.

Si, pour l'os, on peut connaître l'activité cumulée par gramme de tissu, il n'en va pas de même pour les cancers «sanguins», qui sont en majorité des lympho-réticulo-sarcomes d'origine extra-thoracique. Il faut noter que les lympho-réticulo-sarcomes dont le point de départ est intra-thoracique ont une chronologie d'apparition beaucoup plus tardive, comparable à celle des carcinomes pulmonaires des animaux ayant reçu la même dose.

Tissus mous — Le tableau III résume les données sur les cancers des tissus mous observés chez les animaux contaminés. Ces cancers sont d'apparition plus tardive que les cancers du poumon.

Des cancers différents ont été observés sur les mêmes rats et le tableau IV résume les observations.

TABLEAU III. MORTALITE CUMULEE DES RATS AYANT UN CANCER DES TISSUS MOUS

	Jours					
	< 450	< 500	< 550	< 600	< 650	< 700
f N	2	5	10	10	20	28
%	8	19	38	38	79	100

Témoins — Le tableau V résume les données sur la survie des rats témoins ayant présenté un cancer. Un seul animal en a présenté deux.

La figure 4 regroupe toutes les données et montre que les différents cancers n'ont pas, dans le même groupe d'animaux, la même chronologie d'apparition.

2. RELATION ENTRE LA DOSE ET LA FREQUENCE DES CANCERS

L'irradiation, outre l'apparition de cancers, entraîne un raccourcissement de la durée de vie. Les cancers apparaissant chez les animaux ayant la plus longue survie, nous n'avons tenu compte pour les comparer aux cancéreux que des rats sans cancer ayant eu une survie au moins égale à celle du premier rat cancéreux observé ou supérieure au temps nécessaire pour l'apparition des cancers chez les témoins.

Poumons — Le tableau VI résume les résultats pour les deux modalités de contamination pulmonaire. Il montre qu'à activités cumulées égales, les éléments diffusibles induisent proportionnellement plus de cancers que les éléments peu diffusibles. Ceci peut être dû à leur répartition plus homogène qui atteint plus de cellules et au fait que, la dose étant délivrée en un temps plus court, plus de tumeurs ont le temps d'apparaître pour des temps de latence comparables.

Os — Le tableau VII résume les résultats. Il montre qu'à activités cumulées comparables, la fréquence des ostéo-sarcomes est inférieure à celle des cancers du poumon, mais dans le poumon plusieurs lignées cellulaires sont susceptibles d'être atteintes, ce qui augmente le risque pour cet organe.

Tissus mous — Le tableau VIII résume les résultats. Il montre qu'à faibles doses on a une augmentation de fréquence de nombreux cancers. Si, pour chaque lignée, la probabilité reste faible, la multiplicité des lignées atteintes permet la mise en évidence de l'effet.

Tissus «sanguins» — On ne peut calculer la dose aux tissus «sanguins». Mais, connaissant l'activité déposée pulmonaire initiale et la fraction qui en diffuse, on peut comparer les animaux. Les cancers «sanguins» apparaissent chez des animaux dont l'«imprégnation» radioactive est inférieure à celle des rats porteurs d'ostéo-sarcomes. Nos résultats confirment donc bien la grande sensibilité de ce tissu.

TABLEAU IV. NOMBRE DE LIGNEES CANCEREUSES OBSERVEES CHEZ UN MEME RAT

Poumons seuls (Nombre de rats)	1 lignée 136	2 lignées 39		
Poumons et autres cancers extra-pulmonaires (Nombre de rats)	Poumon seul 150	Poumon + 1 lignée 21	Poumon + 2 lignées 5	Poumon + 3 lignées ou plus 1
Lignées cancéreuses extra-pulmonaires sans le poumon	1 lignée 22	2 lignées 4		

TABLEAU V. MORTALITE CUMULEE DES RATS TEMOINS AYANT UN CANCER

	Jours					
	< 600	< 650	< 700	< 750	< 800	< 850
	f N	f N	f N	f N	f N	f N
	4	7	8	-	9	10
%	40	70	80	-	90	100

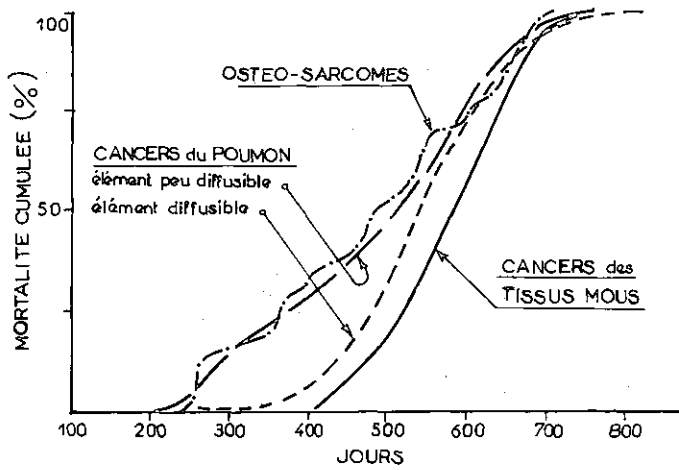


FIG. 4. Mortalité cumulée des rats ayant un cancer du poumon, un ostéo-sarcome ou un cancer des tissus mous, en fonction du temps de survie.

TABLEAU VI. RELATION ENTRE L'ACTIVITE CUMULEE ET LA FREQUENCE DES CANCERS DU POUMON

Eléments non diffusibles	Activité cumulée ($\times 10^3$ α /g de poumon frais)		
	10 - 15	20 - 40	80 - 120
N cancers	12	61	38
N rats	21	69	36
%	57	88	106
Eléments diffusibles	2 - 10	10 - 20	20 - 50
			80
N cancers	20	43	40
N rats	41	50	38
%	49	86	105

TABLEAU VII. RELATION ENTRE L'ACTIVITE CUMULEE ET LA FREQUENCE DES OSTEO-SARCOMES

	Activité cumulée ($\times 10^3$ α /g de tissu frais)		
	1 - 5	5 - 10	10 - 15
N cancers	14	5	9
N rats	83	20	21
% cancers	17	25	43

TABLEAU VIII. RELATION ENTRE L'ACTIVITE CUMULEE ET LA FREQUENCE DES CANCERS DANS LES TISSUS MOUS

	Activité cumulée ($\times 10^3 \alpha/g$ de tissu frais)		
	0,005 - 0,03	0,03 - 0,09	0,09 - 0,27
N cancers	6	7	10
N rats	45	17	19
% cancers	13	41	53

TABLEAU IX. NOMBRE DE CANCERS PULMONAIRES EN FONCTION DES LIGNEES CELLULAIRES ET DE LA DIFFUSIBILITE DES CONTAMINANTS

	Eléments non diffusibles		Eléments diffusibles	
	Sprague Dawley	Wistar	Sprague Dawley	Wistar
Bronchogéniques	41	16	26	21
Bronchiolo-alvéolaires	37	12	23	23
Sarcomes	2	4	1	10

TABLEAU X. RELATION ENTRE LE NOMBRE DE CANCERS EXTRA-PULMONAIRES ET LES DIFFERENTS TYPES DE LIGNEES CELLULAIRES

	Nombre de cancers	Type de lignées cellulaires
Tissu osseux	28	1
Tissu hématopoïétique	10	2
Tissus mous	26	12

3. ANALYSE DES RESULTATS OBTENUS DANS LES DIFFERENTS ORGANES

Poumons — Si on classe les cancers observés en trois groupes: carcinomes bronchogéniques, carcinomes à pneumocytes II et sarcomes, on obtient les résultats regroupés dans le tableau IX. Ils montrent que, malgré des territoires de rétention pulmonaire différents, on n'observe pas, pour une même race de rats, de différences entre les éléments diffusibles et peu diffusibles. D'autres expériences, pratiquées sur des rats Sprague Dawley avec du radon-222 [3, 4], dont les produits de filiation se déposent principalement dans l'arbre trachéo-bronchique, ont fourni les résultats suivants: bronchogéniques = 70, bronchiolo-alvéolaires = 62, sarcomes = 3.

Tous ces résultats montrent clairement que le type histologique ne dépend pas de l'irradiation locale de l'organe, mais est sous la dépendance

de la sensibilité particulière d'une des lignées cellulaires pulmonaires. Cette conclusion est en accord avec les résultats présentés à ce colloque par Nolibé et al. [5].

Autres organes – Le tableau X résume les résultats.

Synthèse – Si on divise la fréquence des cancers observés par le nombre de lignées atteintes en tenant compte, dans le cas du poumon, de la sensibilité de chacune des trois lignées, on peut regrouper les résultats (fig.5).

4. MECANISME POSSIBLE D'INDUCTION DES CANCERS PAR LES EMETTEURS α

Ce mécanisme doit tenir compte du fait qu'il existe un temps de latence qui, pour une même espèce, dépend de la dose. Jacobi a calculé, pour les ostéo-sarcomes humains et expérimentaux, la relation dose-fréquence en tenant compte du temps de latence [6]. Si on généralise ses hypothèses de départ, on peut penser que toute lignée cellulaire deviendrait un jour cancéreuse si l'organisme qui l'héberge avait une durée de vie suffisante. Dans un groupe d'animaux, ce temps est réparti suivant une loi statistique. L'irradiation ne fait qu'accélérer ce phénomène et cette accélération est d'autant plus importante que la dose est plus forte. Dans un groupe dont les poumons ont été irradiés à une même dose, chaque individu peut, soit avoir un cancer pulmonaire, soit mourir «normalement», suivant la sensibilité particulière de son poumon et la cinétique de son «vieillessement».

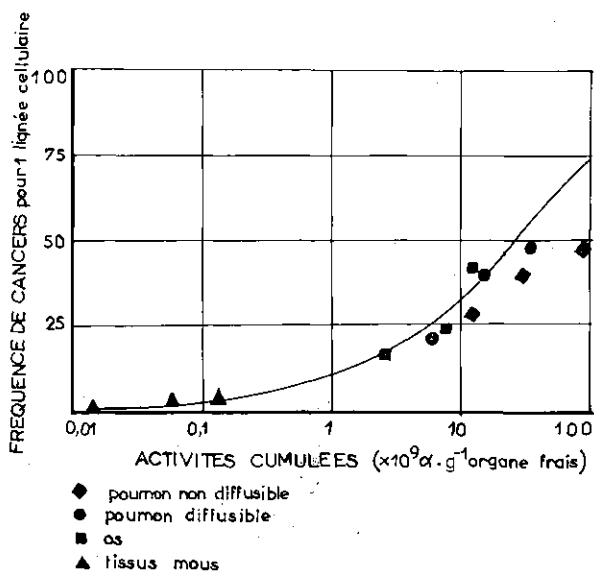


FIG. 5. Relation dose cumulée - fréquence de cancers pour une seule lignée cellulaire: ◆ poumon, non diffusibles; ● poumon, diffusibles; ■ os; ▲ tissus mous.

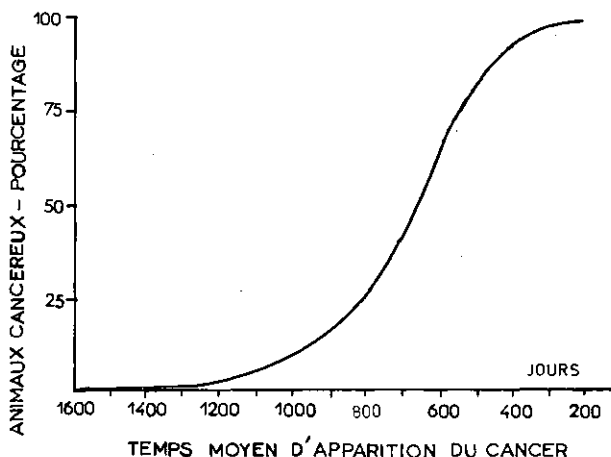


FIG. 6. Relation théorique entre la fréquence des cancers et le temps moyen séparant la contamination et l'apparition des cancers. La survie moyenne des rats non contaminés est de 750 jours après la contamination.

On peut tracer une courbe reliant la fréquence théorique des cancers des poumons et le raccourcissement de la moyenne des temps auxquels ces cancers apparaissent dans un groupe d'animaux. La figure 6 montre que cette relation théorique est de forme sigmoïde.

Si l'on connaissait la relation entre la dose et la valeur de la moyenne des temps auxquels apparaissent les cancers, on pourrait calculer la relation entre la dose et la fréquence des cancers.

Si l'on ajuste la courbe de la figure 6 avec les points expérimentaux de la figure 5, on voit que le raccourcissement de ce temps est sensiblement proportionnel au logarithme de la dose mais que ceci n'est vrai que pour les faibles doses car, à des niveaux plus élevés, d'autres phénomènes empêchent l'apparition des tumeurs.

Une relation proportionnelle entre le logarithme de la dose et le temps de survie des animaux existe aussi chez ceux qui ont été contaminés à très hauts niveaux [7]. Il est donc possible que ce soit un même mécanisme qui intervienne à tous les niveaux de dose, mécanisme qui ne serait qu'une accélération de processus naturels. L'apparence stochastique de l'effet n'est alors due qu'à la forme de la répartition statistique dans un groupe de la sensibilité propre de chacun des individus.

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BIOLOGICAL EFFECT OF FOCAL ALPHA RADIATION ON THE HAMSTER LUNG *

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Abstract

BIOLOGICAL EFFECT OF FOCAL ALPHA RADIATION ON THE HAMSTER LUNG.

Monodispersed 10- μ m-diameter ZrO_2 ceramic microspheres, containing varying amounts of $^{239}PuO_2$ or $^{238}PuO_2$, were injected into the jugular vein of 100-day-old Syrian hamsters. These biologically inert microspheres lodged subsequently in pulmonary capillaries and remained static in position throughout the lifespan of the animals with no discernible inflammatory response. The numbers of microspheres injected ranged from 2000 to 10 000 and the specific activity from 0 to 59 pCi/sphere so that the lung burdens were 0 to 354 nCi/animal. At these numbers, each plutonium-laden microsphere served as an independent focal source of α radiation. No consistent alteration of lifespans post-exposure was seen in the experimental hamsters compared with controls. Pulmonary tissue responses were minimal, with only 0.5% of the animals given Pu/ ZrO_2 microspheres ultimately developing primary tumours of the lung. No unexpected gross or histologic lesions were found in other major body tissues.

1. INTRODUCTION

Paramount in the consideration of expanded use of nuclear energy is an understanding of the influence of plutonium and the other transuranic elements on mammalian systems. The impact of radioactive emissions from these elements on the respiratory tract is especially relevant, as the most probable mode of accidental human exposure would be via inhalation of insoluble radionuclide particles. Such particles might constitute focal sources of intense radiation. These "hot spots" would subsequently subject adjacent pulmonary epithelium to doses of radiation that would far exceed those resulting from the same total activity distributed homogeneously in the pulmonary milieu. Whether the lung is more susceptible to insult by isolated "hot spots" or by diffuse radiation is as yet conjectural and has been the subject of considerable debate and controversy.

A thorough understanding of radionuclide-induced disease, including tumorigenic proclivities, is absolutely essential if meaningful guidelines for potential human exposures are to be established. While there are sound epidemiological data available linking exposure to alpha radiation with an increased frequency of lung cancer in man [1-3], precise information relative to this problem can be obtained only through experimental means in the laboratory. Yet the biological and physical complexities of radiation-tissue interactions are so profound that available experimental data have not provided definitive answers or resolved the issue regarding local vs diffuse radiation hazards.

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Tumorigenesis is an extremely subtle and complicated process even in the context of biological complexities; the mechanisms involved have not been elucidated and are being pursued assiduously. That external and internal irradiation will induce cancer is well established. Among the internally deposited radionuclides, plutonium, an alpha emitter, is accepted as one of the most hazardous radiation sources *in vivo*. Particulate plutonium has been demonstrated to have substantial pulmonary carcinogenic propensities in several species, including beagle dogs [4], rats [5], mice [6] and baboons [7]. Polonium-210, also an alpha emitter, has been found to be a potent tumorigenic agent in the respiratory tract when given either as a particulate aerosol in rats [8] or in solution instilled intratracheally in hamsters [9].

We have initiated a series of experiments to resolve some of the questions regarding focal vs diffuse radiation insult of pulmonary tissue and to examine the effects of alpha radiation, devoid of chemical and physical irritability, on the hamster respiratory tract. Syrian hamsters (*Mesocricetus auratus*) were selected because of their special fitness for the study of pulmonary carcinogenesis [10] and established susceptibility to alpha radiation-induced respiratory tumorigenesis [9]. This presentation will focus on the detailed results of a study of approximately 1000 animals that received ^{238}Pu or ^{239}Pu incorporated in zirconium oxide (ZrO_2) microspheres injected intravenously so that they lodged in the pulmonary capillaries. Information regarding other aspects of the project is available elsewhere [11-13].

2. MATERIALS AND METHODS

For this facet of the total project, Syrian hamsters were exposed to ^{238}Pu or ^{239}Pu alpha radiation when 100 days old. The animals were obtained when 4- to 6-weeks old from the Lakeview Hamster Colony in Newfield, New Jersey. They were housed two to a polycarbonate cage containing low-dust factor aspen shavings and were suspended on aluminum shelves covered with spun polyester filters (DuPont #22 Spinbonded Polyester Filter, E. I. DuPont Company, Wilmington, Delaware). Cages and bedding were changed twice a week. The hamsters were fed a commercial stock diet (Teklad Hamster Diet®[®], Teklad Mills, Winfield, Iowa) and were given chlorinated water *ad libitum*.

After the conditioning period, when the hamsters were approximately 100-days old, they were assigned to either one of ten experimental or two control groups (Table I). One of the control groups received no additional treatment, while the other was given 2000 ZrO_2 microspheres containing no plutonium.

Hamsters in the experimental groups were given either 2000, 6000 or 10 000 monodispersed 10- μm diameter ZrO_2 ceramic microspheres containing varying amounts of ^{238}Pu or ^{239}Pu .

Total lung burdens included the range in which tumor induction was noted from soluble ^{210}Po experiments. Details of microsphere production and plutonium incorporation are given elsewhere [14]. Briefly, an aqueous colloidal solution of ZrO_2 , to which a small amount of $\text{Pu}(\text{NO}_3)_4$ has been added, was injected as a 40- μm stream into a 1-mm stream of 2-ethylhexanol in coaxial, laminar flow. A sonic oscillation broke the stream into uniform droplets which were collected in a large volume of 2-ethylhexanol; the droplets gelled and were then filtered for further dehydration and final firing at 1000°C. Measurements of the distribution of sphere volumes gave a coefficient of variation of 4% (corresponding to 1.3% in diameter), while the variability in plutonium content was established to be 2.6%. Because of the similarity of PuO_2 to ZrO_2 , the plutonium was probably present in the microspheres as a solid solution.

TABLE I. SUMMARY OF EXPOSURES OF HAMSTERS TO INTRAVENOUS PLUTONIUM MICROSPHERES

Number of Animals	Microspheres per Animal	Specific Activity (pCi/sphere)	Lung Burden (nCi)	Plutonium Isotope	Median Life Spans Post-injection (days)
71	0	-	-	-	610
68	2000	0.00	0.00	-	528
68	2000	0.07	0.14	^{239}Pu	476
71	2000	0.22	0.44	^{239}Pu	659
71	2000	0.42	0.84	^{239}Pu	542
74	2000	0.91	1.82	^{239}Pu	612
71	2000	4.30	8.60	^{238}Pu	518
70	2000	13.30	26.00	^{238}Pu	495
72	2000	59.00	118.00	^{238}Pu	494
47	10,000	0.22	2.20	^{239}Pu	624
154	6000	4.30	26.00	^{238}Pu	622
148	6000	59.00	354.00	^{238}Pu	534

To facilitate the measurements of injected and retained doses *in vivo*, a low-level tag of ^{57}Co was added to all spheres. Cobalt-57 decays by pure electron capture with the emission of gamma rays of energy 122 and 136 keV but no charged particles. The level added (~ 0.5 pCi/sphere) contributed a dose to the hamster (~ 1 mr/yr) which is considerably less than natural background (~ 150 mr/yr at an altitude of 2200 m in Los Alamos, New Mexico, U.S.A.)

To deliver the plutonium-laden ZrO_2 microspheres, hamsters were anesthetized intraperitoneally with pentobarbital sodium (Nembutal[®], Abbott Laboratories, North Chicago, Illinois), placed in dorsal recumbency, and either jugular vein exposed by surgical incision and blunt dissection after skin disinfection. After injection, the wounds were closed with 9-mm wound clips (Autoclips[®], Clay-Adams, New York City, New York). Because the $\text{ZrO}_2/\text{PuO}_2$ microspheres had a high density, a rapidly pulsed dental cleaning device (Water-Pik[®], Aqua Tec Corporation, Denver, Colorado) was used to inject the microspheres, suspended in 0.2 ml 0.15 M NaCl, into the isolated jugular vein [15]. Using this procedure, the radioactivity remained quantitatively in the lung [12].

All animals were checked two or three times daily for the duration of their life spans, moribund hamsters were killed and necropsied, and dead hamsters were necropsied immediately. The respiratory tract was inflated via the trachea with 5 to 6 cm^3 of 10% neutral buffered formalin and the trachea ligated. The respiratory tract was removed *en bloc*, fixed in 10% neutral buffered formalin, and counted for radioactivity with twin sodium iodide crystals.

The five lung lobes were separated after fixation, processed by standard methods, and routinely stained with hematoxylin and eosin. The lungs were also examined autoradiographically for alpha tracks. Likewise,

complete microscopic examination was done with samples of liver, kidney, adrenal, stomach, small intestine, large intestine and gonads. Special histochemical stains were used when appropriate.

3. RESULTS

3.1. Microspheres and radiation

When serial sections of the lung were examined microscopically, the microspheres were distributed randomly throughout the capillary bed. One or two microspheres were usually found per section of lung tissue. They usually occurred singly, entrapped in interalveolar septa; clusters of two to four microspheres were noted occasionally in the septa. Microspheres were frequently noted in capillaries adjacent to bronchial epithelium. That portions of bronchial epithelium were exposed to plutonium alpha radiation was also established autoradiographically.

The biologically inert microspheres did not initiate any inflammatory reaction as long as they remained static in the capillaries. Slight foreign-body tissue reactions, consisting of focal aggregations of alveolar macrophages, were observed only when a microsphere was extruded into an alveolar space -- an uncommon occurrence.

With the relatively small number of microspheres injected in these animals, there was little, if any, overlapping of individual radiation fields. Approximately 1% of the total lung was irradiated in those hamsters receiving 2000 microspheres, 3% with 6000 microspheres, and 5% with 10 000 microspheres. Ninety-five \pm 3% (S. E.) of the radioactive material injected remained in the respiratory tracts of all groups at necropsy.

3.2. Life spans

Median life spans post-injection are included in Table I. They ranged from a low of 476 d to a high of 659 d in those animals that received the plutonium-laden microspheres. Control animals receiving no microspheres had a median life span post-exposure of 610 d and the group given 2000 microspheres composed of only ZrO_2 528 d. Increasing plutonium burdens did not consistently affect the life spans of experimental animals compared to the controls.

3.3. Non-neoplastic pulmonary lesions

Osseous metaplasia was found in all groups at an incidence of 2 to 28%, with the highest frequency seen in control animals. Likewise, focal accumulation of hemosiderosites was noted in control and experimental animals, with a slightly higher but not consistently significant incidence in experimental hamsters. Pneumonia was found in about 5% of control and experimental animals.

3.4. Pre-neoplastic and neoplastic pulmonary lesions

Bronchiolar adenomatoid lesions (BAL), proliferations of terminal bronchiolar epithelium that are considered by many to be neoplastic precursors, were limited to groups receiving "hot" microspheres (Table II). It is noteworthy to mention that the group of hamsters with the highest BAL frequency, 6% (6000 microspheres, 26 nCi lung burden), did not develop even a single primary lung tumor (Table III).

Primary lung tumors were clustered in two groups of experimental animals, as noted in Table III. Two animals receiving 2000 microspheres, with a lung burden of 0.84 nCi, developed tumors: one a hemangiosarcoma of the left lung, and the other a well circumscribed adenoma also originating

TABLE II. DISTRIBUTION OF BRONCHIOLAR ADENOMATOID LESIONS (BAL)

Number of Animals	Microspheres per Animal	Microsphere Activity (pCi/sphere)	Lung Burden (nCi)	Plutonium Isotope	BAL Incidence
71	2000	0.22	0.44	²³⁹ Pu	1 (1%)
71	2000	0.42	0.84	²³⁹ Pu	2 (3%)
71	2000	4.30	8.60	²³⁸ Pu	2 (3%)
47	10 000	0.22	2.20	²³⁹ Pu	1 (2%)
154	6000	4.30	26.00	²³⁸ Pu	9 (6%)
148	6000	59.00	354.00	²³⁸ Pu	3 (2%)

TABLE III. PRIMARY TUMORS OF THE LUNG

Number of Animals	Microspheres per Animal	Microsphere Activity (pCi/sphere)	Lung Burden (nCi)	Plutonium Isotope	Exposure Time (weeks)	Tumor
71	2000	0.42	0.84	²³⁹ Pu	41	Hemangiosarcoma
71	2000	0.42	0.84	²³⁹ Pu	62	Adenoma
148	6000	59.00	354.00	²³⁸ Pu	104	Mucinous adenocarcinoma
148	6000	59.00	354.00	²³⁸ Pu	115	Mucinous adenocarcinoma

in the left lobe of the lung. Two mucinous adenocarcinomas were found in the group that received 6000 microspheres, with a lung burden of 354 nCi: one in the peripheral left lung, and the other arising in the right anterior lobe.

3.5. Extrapulmonary lesions

No unexpected changes were found upon gross and histologic examination of other major body tissues.

4. DISCUSSION

Under the described experimental conditions, even large amounts of plutonium alpha radiation deposited in the lungs did not predictably or consistently alter the life spans of experimental animals compared to controls. Simplistic as this observation might be, nonetheless it is significant relative to the issue at hand (i.e. what are the life-threatening hazards of internally deposited alpha radiation?).

An unexpected but fascinating observation was the minimal pulmonary carcinogenicity of Pu/ZrO₂ in these animals. This is in distinct contrast to the marked tumorigenicity of plutonium for the lung in other species [4-7]

but is in close agreement with preliminary reports from other laboratories studying the effects of plutonium aerosols on the hamster respiratory tract [16,17]. This finding is perplexing in light of the finding that even small amounts of ^{210}Po alpha radiation readily induce respiratory-tract tumors in hamsters [9] and that lung tumors appear in hamsters a few weeks after the intratracheal administration of moderate amounts of benzo(a)pyrene [18].

A factor or factors requisite for tumor initiation and development obviously were lacking in this study. The microspheres, being statically lodged in capillaries and devoid of chemical or physical irritability, may not have had sufficient carcinogenic and/or cocarcinogenic stimuli to induce a significant number of lung tumors. Another consideration was the paucity of systemic involvement as the radiation sources were permanently restricted to the lung. Beagle dogs, exposed to high doses of ^{239}Pu aerosols, that later developed primary lung tumors, had only a small fraction of the initial ^{239}Pu alveolar deposit remaining in their respiratory tracts. The remaining ^{239}Pu was found translocated to thoracic lymph nodes, liver, skeleton, and abdominal lymph nodes [4]. A suppression of humoral and cellular defense mechanisms may play a prominent role as to which cells are transformed and allowed to manifest ultimately as tumors.

In summary, these findings do not add credence to the supposition that tumor induction and expression can be predicted solely on the number of cells at risk. Also, discrete focal alpha radiation alone was not an efficient respiratory carcinogen in the hamster.

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DISCUSSION

D. NOLIBE: In these animals, did you observe any changes in the blood lymphocyte population?

D.M. SMITH: We were unable to demonstrate any effect on the circulating leucocyte, including lymphocyte, population.

R.O. MCCLELLAN: Have you observed any neoplasms in the lungs of control animals or animals which received "cold" ZrO_2 microspheres?

D.M. SMITH: No primary lung tumours were noted in these groups of control animals.

R.O. MCCLELLAN: Have you had the opportunity to evaluate the appearance of the adenomatoid lesions in a function of time?

D.M. SMITH: Yes, generally speaking, BAL tended to develop late in the animals' lifespans. However, it was observed occasionally in animals that died shortly after exposure.

R.O. McCLELLAN: Recognizing that some persons might compute the probability of lung cancer per particle, i.e. lung cancers/ 10^6 Pu particles, I wonder whether you have made such calculations. And would you please comment on the appropriateness of such calculation for risk estimation purposes?

D.M. SMITH: We have indeed made those calculations, and the predicted number of tumours - if one subscribes to such an approach - was far greater than the number which actually developed. Our data do not add credence to this method of establishing minimum permissible exposure standards.

M. GOLDMAN: Did you perform radiochemical verification to show that no ^{238}Pu or ^{239}Pu was leached off the spheres and redeposited in other tissues?

D.M. SMITH: Leaching would not be expected because of the insolubility of ZrO_2 and PuO_2 and because the latter is present in solid state throughout the entire sphere at a low chemical concentration (less than 1%). We have made γ measurements on necropsy samples, dividing the hamster into lung, liver and carcass, and find over 90% of the activity in the lung a year or more after injection.

Miriam FINKEL (Co-chairman): This is one of the most important experiments to date in radiation oncogenesis because it shows quite convincingly that radiation acting alone is not oncogenic. Have you considered the possibility that chemical toxicity is important in the induction of tumours by plutonium?

D.M. SMITH: The chemistry of the radioelement is undoubtedly important, especially in determining the target organ. Under the conditions of this experiment, plutonium exerted its influence on pulmonary tissue via α particles alone since the ceramic microspheres were chemically and physically inert. We have initiated a series of experiments to study this fascinating aspect of "radiation" carcinogenesis.

However, it should be pointed out that some authorities feel that direct chemical toxicity is unlikely. For one thing, tumorigenesis has been observed with infinitesimal amounts of material. Thus 1000 nCi of ^{210}Po corresponds to 4.5×10^{-12} g of Po but is highly tumorigenic when instilled into hamster lungs. Soviet researchers have observed lung tumours with a few micrograms of ^{239}Pu compounds, and Sanders has found comparable carcinogenicity with nanogram amounts of ^{238}Pu . If chemical effects were involved, the lower specific activity ^{239}Pu would seem more effective when calculated purely on a radiation basis, whereas the opposite appeared to be true.

J.C. NENOT: Do you think there is a relationship between this extremely low cancer rate and the absolute immobility of the plutonium particle in the tissue?

D.M. SMITH: The immobility of the microspheres under these experimental conditions is probably a very important factor in the lack of pulmonary carcinogenesis. To help resolve the question, we have initiated a series of experiments in which the spheres are being administered intratracheally. With this form of delivery, the spheres should not be immobile in the lung.

C.W. MAYES: I think that, in order to settle the question of why plutonium microspheres introduced intravenously do not cause appreciable lung cancer in hamsters, we must also consider the inhaled plutonium.

D.M. SMITH: Yes, that is an important consideration since the IU_1 spheres are biologically inert and statically lodged in the pulmonary capillaries. Inhaled plutonium may very well enhance tumorigenesis for several reasons. For example, it would add chemical and physical irritability factors, and the plutonium would undoubtedly be translocated to other body tissues, resulting in systemic involvement.

EFFECT OF SIZE AND ALPHA FLUX OF $^{239}\text{PuO}_2$ PARTICLES ON PRODUCTION OF CHROMOSOME ABERRATIONS IN LIVER OF CHINESE HAMSTER

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Abstract

EFFECT OF SIZE AND α FLUX OF $^{239}\text{PuO}_2$ PARTICLES ON PRODUCTION OF CHROMOSOME ABERRATIONS IN LIVER OF CHINESE HAMSTER.

Two important variables in determining the biological effect of α -emitting radioactive particles, "hot particles", are the flux of α emissions per particle and the fraction of the cell population exposed. To generate a spectrum of these conditions, Chinese hamsters were injected intravenously with $^{239}\text{PuO}_2$ or $^{238}\text{PuO}_2$ particles or with ^{239}Pu or ^{238}Pu citrate. The plutonium was retained with a long effective half-life in the liver for both the citrate and oxide forms. The particles of $^{239}\text{PuO}_2$ tended to aggregate as a function of time after injection. The level and distribution of chromosome damage in liver cells were determined as a function of the dose, fraction of liver exposed and α flux per particle. Aberration frequency following injection of ^{239}Pu and ^{238}Pu citrate increased with slopes of 4.8×10^{-3} and 3.8×10^{-3} aberrations/cell/rad, respectively. This rate was about three times higher than observed for $^{239}\text{PuO}_2$ particles and ten times as high as observed following injection with the smallest $^{239}\text{PuO}_2$ particles. When aberration frequency was related to average dose there was little effect of change in α flux until it reached a value of 860 disintegrations/particle/day. Above this level, there was a marked decrease in the aberration frequency per rad dose. Evidence of cell killing and extreme amounts of chromosome damage was found in liver cells after injection of $^{238}\text{PuO}_2$ and $^{239}\text{PuO}_2$. Increasing the percentage of the liver irradiated increased the amount of chromosome damage produced. More cells are at risk for cellular damage with uniformly distributed plutonium, and those cells in close proximity to plutonium particles may be lethally irradiated and would not be at risk for tumour production.

INTRODUCTION

Large quantities of ^{239}Pu are expected to be used as a fuel in breeder reactors. At all stages of the fuel cycle ^{238}Pu , which is approximately 280 times higher in alpha specific activity than ^{239}Pu , will also be present. At various stages of the fuel cycle, other high specific activity radionuclides, such as ^{244}Cm , will also be present. If an aerosol were produced from reactor fuel in a nuclear accident, the size, specific activity and alpha flux of the particles may determine the nature and extent of cellular damage and ultimately the potential for cancer induction.

Calculation of radiation dose from these particles can be carried out at any level of biological organization. For a given deposited activity, the dose derived is dependent on the volume of tissue of interest and the distribution

and emission of the isotope. When an alpha-emitting particle is deposited in soft tissue, especially lung, the cells close to the particle receive a large radiation dose whereas the average dose to the lung may be small. On the other hand, if the activity present in the particle were distributed throughout the lung, the dose to any cell may be small but the number of cells "hit" by an alpha particle would be large. Calculations [1,2] and experimentation [3] on which of these dose distributions has the greatest hazard associated with it have been made and are not in agreement. Data which help us understand this important question will play a major role in standard setting for alpha-emitting radioactive materials.

In the present report, the cellular damage produced in the liver by injection of Chinese hamsters with $^{239}\text{PuO}_2$, $^{238}\text{PuO}_2$, ^{239}Pu citrate or ^{238}Pu citrate has been determined. Chromosome aberration frequency has been used as an index of cellular damage and provides a model to aid in understanding the amount and distribution of cellular damage after exposure to a range of dose patterns. By using $^{239}\text{PuO}_2$ and $^{238}\text{PuO}_2$ particles of different sizes, a wide range of specific activity and alpha flux per particle have been spanned. The distribution of dose can be related to the distribution of chromosome damage and the number of cells damaged can be estimated.

METHODS

Polydisperse spherical $^{238}\text{PuO}_2$ and $^{239}\text{PuO}_2$ particles were produced by the method described by Kotrappa et al. [4]. The polydisperse distribution of particles was then separated into narrow ranges of particle sizes using a Lovelace Aerosol Particle Separator [5]. The particles were washed and centrifuged to minimize the amount of soluble material injected. The particles were then suspended in distilled water with either 0.2% Dowfax or dipalmitoyl lecithin, surface active agents which were used to minimize particle adhesion on the walls of injection vials and clumping in solution. A volume of 0.1 ml of this mixture was injected in the jugular sinus of the hamsters. Additional animals were injected with ^{239}Pu or ^{238}Pu citrate to produce a uniform dose distribution. Control animals were injected with Na citrate or distilled water with a surface active agent. The experimental design is illustrated in Table I. Particle sizes used, activity injected per gram body weight and times of sacrifice are shown. There were three different particle sizes used in the $^{238}\text{PuO}_2$ experiment with four or five different activity levels per particle size. All animals were sacrificed at an early time after injection. Animals were injected with four different sizes of ^{239}Pu particles; three of the sizes were injected

Table I
Experimental Design for Determining the Effect of Alpha Flux and Dose
Distribution on the Frequency of Chromosome Aberrations
in the Chinese Hamster Liver

Isotope and Chemical Form	Particle Size	Injected Activity ($\mu\text{Ci/g}$)	Sacrifice Time (days)				
			6	10	15	42	120
^{239}Pu citrate	Ionic	6×10^{-4}	X		X	X	X
^{238}Pu citrate	Ionic	5×10^{-3}		X			
$^{239}\text{PuO}_2$	0.15	1×10^{-3}	X		X	X	
		1×10^{-3}	X		X	X	
		1×10^{-3}	X		X	X	
		6×10^{-2}	X		X	X	X
		2×10^{-3}	X		X	X	X
		6×10^{-4}	X		X	X	X
		2×10^{-4}	X		X	X	X
		6×10^{-5}	X		X	X	X
$^{238}\text{PuO}_2$	0.17	2×10^{-2}		X			
		5×10^{-3}		X			
		5×10^{-4}		X			
		2×10^{-4}		X			
	0.40	5×10^{-2}		X			
		2×10^{-2}		X			
		7×10^{-3}		X			
		3×10^{-3}		X			
		7×10^{-4}		X			
	1.1	5×10^{-2}		X			
		2×10^{-2}		X			
		5×10^{-3}		X			
		5×10^{-4}		X			

at a single level of activity and the fourth size was injected at five different levels of activity. Animals were injected with a single level of 5×10^{-3} and $6 \times 10^{-4} \mu\text{Ci/g}$ body weight for the ^{238}Pu and ^{239}Pu citrate, respectively. Using these isotopes, activities and particle sizes, a range of specific activities and alpha fluxes was produced. Changes in alpha flux would produce a range of local and average doses to the liver of the hamsters.

Table II
The Effect of Particle Size and Activity on Local Dose Rate and Dose Distribution

Isotope	Particle Size (μm)	Activity ($\mu\text{Ci/g}$ body weight)	Particles/ μCi	Particles/ Liver	Average Dose Rate (rads/day)	Local Dose Rate (rads/day)	Disintegrations/ Day/Particle	Percent of Liver Exposed
$^{239}\text{PuO}_2$	0.15	5×10^{-4}	5.3×10^8	7.2×10^6	2.4	2.4	6.0	100 ⁺
	0.30	5×10^{-4}	9.2×10^7	1.2×10^6	2.4	11	35	21
	0.44	5×10^{-4}	3.1×10^7	4.1×10^5	2.4	32	1×10^2	7.3
	0.84	5×10^{-4}	4.5×10^6	6.1×10^4	2.4	220	7.1×10^2	1.1
$^{238}\text{PuO}_2$	0.17	5×10^{-4}	3.7×10^6	5.0×10^4	2.5	2.8×10^2	8.6×10^2	0.89
	0.41	5×10^{-4}	2.6×10^5	3.5×10^3	2.5	4.0×10^3	1.3×10^4	0.062
	1.10	5×10^{-4}	1.4×10^4	1.9×10^2	2.5	7.8×10^4	2.3×10^5	0.004
^{239}Pu Citrate	Ionic	5×10^{-4}	-	-	2.4	2.4	-	100
^{238}Pu Citrate	Ionic	5×10^{-4}	-	-	2.5	2.5	-	100

Fifty-four (54) hours before sacrifice, the animals had 60% of their liver removed to stimulate cell division. Liver cells were collected in metaphase by injecting colchicine four hours before sacrifice and prepared for chromosome analysis by previously described techniques [6]. All slides were coded and chromosomes scored without the scorer knowing the treatment the cells received.

Autoradiographs and radiochemical analysis were performed at both hepatectomy and sacrifice to determine the amount and distribution of the radioactive material. For animals injected with $^{239}\text{PuO}_2$, the number of alpha tracks in the radiographic emulsion over sections of liver was recorded after three days of exposure. Following $^{238}\text{PuO}_2$ injection, the number of tracks per particle was so great that they could not be counted. However, the number of individual alpha tracks per microscopic field was recorded.

RESULTS

When isotopes were injected as a citrate form, approximately 60% of the sacrifice body burden was retained in the liver with most of the remainder going to the bone. Both organs retained the isotope with a long effective half-life. For the animals injected with $^{238}\text{PuO}_2$ or $^{239}\text{PuO}_2$, 90% of the sacrifice body burden was found in the liver with no evidence of significant loss from liver over the 120 days of this experiment. Retention curves were combined with the activity present at sacrifice and hepatectomy to calculate radiation dose to the liver. Table II was derived to illustrate the dose and dose distribution patterns that could be calculated for the liver, assuming that the range of the alpha particle in soft tissue is 40 μm and that $^{238}\text{PuO}_2$ and $^{239}\text{PuO}_2$ have similar densities of 8.0 and activities of 13.9 and 5.5×10^{-2} Ci/g, respectively. The table was derived using a single injection level of 5×10^{-4} $\mu\text{Ci/g}$ body weight. The interaction of particle size and specific activity produces a range of 7.2×10^6 to 1.9×10^2 particles per liver with the particles giving off from 6.0 to 230 000 disintegrations per day. The local dose rates shown in the table were calculated assuming the energy for each particle was uniformly deposited in a volume of tissue with a radius of 40 μm . The calculated local dose rates varied from 2.4 to 7.8×10^4 rads/day while the average dose calculated on the basis of μCi per gram in the total liver remained at 2.4 to 2.5 rads/day. The volume or percent of the liver irradiated was calculated assuming no overlap or movement of the particles. The experimental data illustrated that this last assumption is not valid because particles aggregate in the liver. The percent of cells at risk for an interaction with an alpha particle ranged from 100 to 0.003%. The animals with

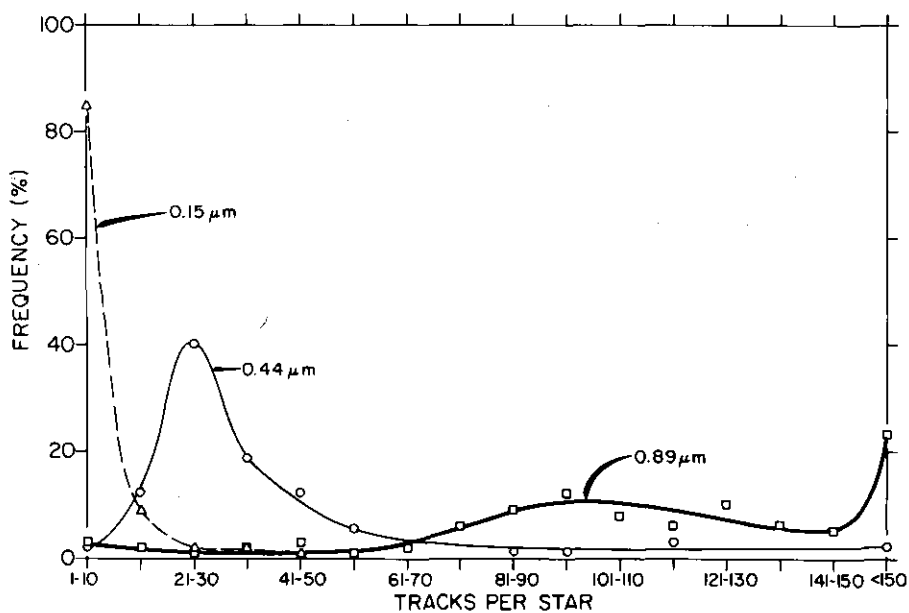


FIG.1. Number of tracks/star observed in autoradiographs of the liver of Chinese hamster 13 days after injection with three different monodispersed $^{239}\text{PuO}_2$ particles.

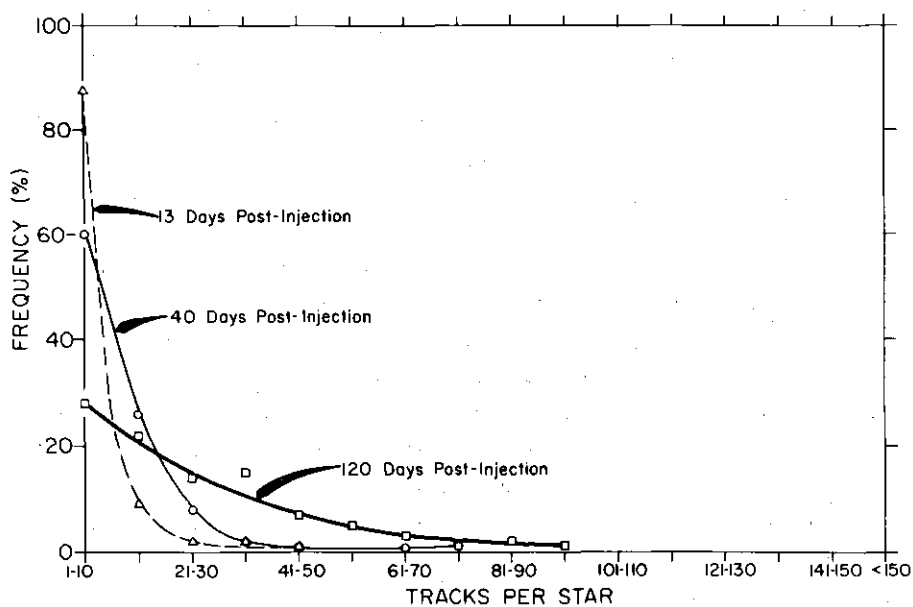


FIG.2. Effect of time on distribution of tracks/star in autoradiographs of the liver following injection with $0.15 \mu\text{m}$ $^{239}\text{PuO}_2$ particles.

the largest local doses had the fewest cells at risk. The table emphasizes how particle size and specific activity influence the dose patterns produced by alpha-emitting particles.

Figures 1 and 2 represent the results of the local dose distribution study for the livers of animals injected with $^{239}\text{PuO}_2$. In these graphs, the number of alpha tracks in the photographic emulsion per $^{239}\text{PuO}_2$ particle is recorded against the frequency of occurrence. Figure 1 shows the three particle sizes at a single time after injection and illustrates that 13 days after injection, the distribution of tracks/star was dependent on particle size with the 0.15- μm particles having over 80% of the stars with less than 10 tracks. In the intermediate size, 0.44 μm , most of the stars had 21-30 tracks with a few having large numbers of tracks. The large particles had a peak in frequency that was rather broad and ranged from 75-130 tracks/star. The difficulty in recording the number of tracks/star in these large particles may be responsible in part for the range of apparent particle sizes.

Figure 2 relates the change in apparent particle size distribution as a function of time between injection and sacrifice for the 0.15- μm particles. The percent of stars with 1-10 tracks dropped from over 80% at 13 days to about 30% by 120 days. This drop was accompanied by an increase in the number and size of the large stars. During this time interval, the total amount of activity present in the liver was very nearly constant. These two facts demonstrate that there is a mechanism in the liver for concentrating the particles into fewer cells. The local dose and effects will then change as a function of time after injection. The same pattern was also observed for the other particle sizes.

The $^{238}\text{PuO}_2$ particles were observed at a single time after injection. Because of the high specific activity of the ^{238}Pu , and the fact that slides were overexposed, it was not possible to do track counts on individual particles. In observing liver slides of animals injected with these particles, it was noted that there were individual alpha tracks in the photographic emulsion and that, for the larger particle sizes, some of the liver cells in nonpartially hepatectomized animals were in mitosis. These observations indicate possible changes in the dose distribution pattern to individual cells and cell killing which may alter the observed dose response of the chromosome aberrations.

Dose response curves for the chromosomes were determined, using the aberration frequency per cell plotted against the average dose to the whole liver (Fig. 3). Both isotopes of plutonium injected in the ionic form had similar dose-response curves with slopes of from $3.8\text{--}4.8 \times 10^{-3}$ aberrations/cell/rad.

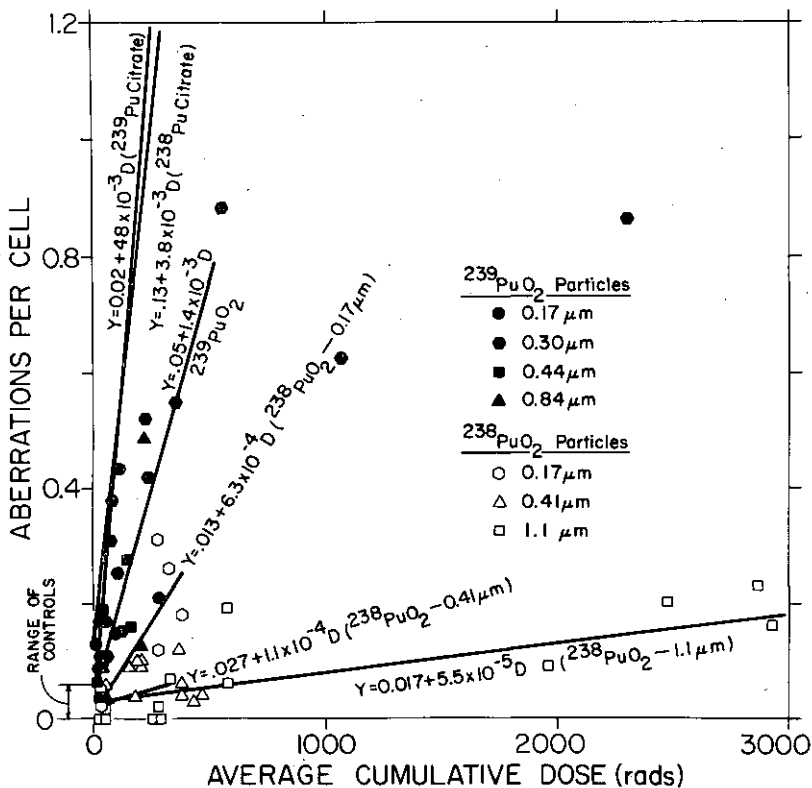


FIG. 3. Effect of α -emitting particle size and specific activity on production of chromosome aberrations in Chinese hamster liver following injection with ^{239}Pu or ^{238}Pu oxide of known size and specific activity.

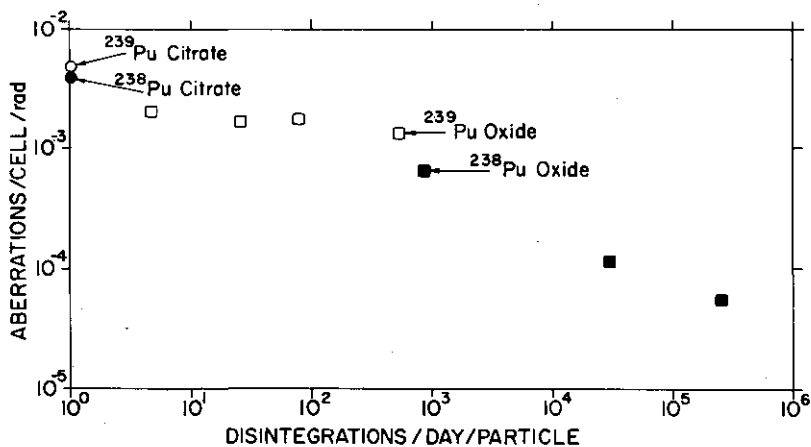


FIG. 4. Effect of specific activity of ^{239}Pu or ^{238}Pu oxide particles in terms of disintegrations/day/particle on frequency of chromosome damage.

This was about three times as high as the 1.4×10^{-3} aberrations/cell/rad observed for the $^{239}\text{PuO}_2$ particles and 10 times higher than the smallest $^{238}\text{PuO}_2$ particles. The larger $^{238}\text{PuO}_2$ particle sizes had dose-response curves with much lower slopes than the citrate.

As an attempt to relate the effect of alpha particle flux and specific activity on the frequency of chromosome aberrations, the slope of the dose-response curve in aberrations/cell/rad was plotted against the number of disintegrations per day per particle (Fig. 4). This figure has the ^{238}Pu and ^{239}Pu citrate plotted as a single point with the values derived at each particle size also plotted. The figure illustrates that as the number of disintegrations per day increases, there is a decrease in the number of aberrations produced per rad of average dose. The ^{238}Pu and ^{239}Pu citrate has the highest efficiency for production of damage.

DISCUSSION

The long-term retention and percent of the sacrifice body burden remaining in the liver of Chinese hamsters injected with plutonium citrate is similar to that observed in dogs [7] and contrasts with the rapid clearance of ^{239}Pu from the mouse liver [8]. This makes the hamster a good small-animal model for studying long-term retention and effects, because the dose rate is almost constant and the dose commitment to the liver can be large. Further, it would appear that this retention pattern is similar to that reported for man [9].

Retention of the particles seemed to be related to phagocytic activity of the Küpffer cells of the liver. For the particle exposure, the most intense irradiation is of these cells with the remainder of the energy being absorbed in the parenchymal cells. The concentration and movement of the $^{239}\text{PuO}_2$ particles is not understood at this time but seems to be related to cell killing with release of the $^{239}\text{PuO}_2$ particles and concentration of the particles in new cells. The rate of cell killing in the $^{239}\text{PuO}_2$ animals seemed to be low and did not result in an increase in mitotic index or a large decrease in aberration frequency.

Although a distributional change in dose pattern was observed as a function of time for the animals injected with $^{239}\text{PuO}_2$, it was not possible to determine a change in the distribution of chromosome damage with time. It has been noted [6] that the distribution of chromosome damage following injection with $^{239}\text{PuO}_2$ was very nonrandom, with some cells containing large amounts of damage. However, changes in distribution of aberrations as a function of particle size have not been possible to observe. This may be related to the fact

that at early times the amount of dose and damage accumulated was small, making a distribution difficult to determine. As doses and time increased, the differences in dose distribution pattern became less as clumping occurred; even the animals injected with small particles had areas of large local dose.

The presence of individual alpha tracks in the animals injected with the $^{238}\text{PuO}_2$ is an indication of breakdown or solubilization of the particles. This has been previously reported [10]. Following injection with the $^{238}\text{PuO}_2$, few cells were observed that had large amounts of chromosome damage. Because the local dose is so high, some cells should have had a high frequency of aberrations. Since this did not occur, it may be that these badly damaged cells are not being scored in the mitotic population. In fact, the aberrations recorded may simply be the result of the soluble component of $^{238}\text{PuO}_2$ present. Figure 4, which plots the aberrations/cell/rad of dose, indicates that at exposure rates above 1000 disintegrations/day there is a sharp decrease in the efficiency of aberration production. This seems to be an indirect measure of either cell death or mitotic delay. The slope of the line in a sense represents the degree to which energy is wasted in lethally irradiated cells. If cell killing or mitotic inhibition occurs when the alpha flux increases above 1000 disintegrations/day/particle, further increase in flux would represent a decrease in the efficiency of chromosome aberration production which is reflected in the slope of the line. Further work on cell killing and turnover as a function of particle size and local dose is necessary to clarify this point. The current research indicates that the amount and distribution of chromosome damage in liver may be useful as an indicator of dose and dose distribution unless local dose is high enough to produce excessive cell killing.

The influence of cell killing and the presence of cells with large amounts of chromosome damage on the risk for tumor induction is still an unanswered question. The energy deposited in these cells may be wasted as far as tumor transformation is concerned; however, the alteration of the local environment by these badly damaged cells may be conducive to tumor growth once transformation of cells has occurred. However, a compilation of data on this "Hot Particle" problem [3] seems to indicate that high local doses are less carcinogenic than uniformly distributed doses which place many cells at risk. Our present data support the concept that a large number of cells at risk with some damage in each, as seen in the exposures to ^{239}Pu or ^{238}Pu citrate, creates a greater amount of chromosome damage and greater potential for cancer production than a large dose to a small population of cells, as is observed with exposure to $^{239}\text{PuO}_2$ or $^{238}\text{PuO}_2$.

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DISCUSSION

Miriam FINKEL (Co-chairman): An excellent comparative study would be to use the Los Alamos plutonium beads deposited in the liver of your Chinese hamsters. Perhaps it could then be determined whether the chromosomal changes which you see are induced by radiation alone. Have you thought of performing such a study?

A.L. BROOKS: As regards local dose distribution patterns, we can generate patterns similar to those seen in the Los Alamos studies, using

our smaller $^{239}\text{PuO}_2$ or $^{238}\text{PuO}_2$ particles. The advantage of the study which you are suggesting would be that the particles would not move around in the liver and local dose characterization would thus be made easier. I would predict, however, that the results in terms of the relationship between the dose distribution and the frequency and distribution of chromosome aberrations would be similar to those observed in the present study.

BIOLOGICAL EFFECTIVENESS OF ^{239}Pu , ^{144}Ce AND ^{90}Sr CITRATE IN PRODUCING CHROMOSOME DAMAGE, BONE-RELATED TUMOURS, LIVER TUMOURS AND LIFE SHORTENING IN THE CHINESE HAMSTER

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Abstract

BIOLOGICAL EFFECTIVENESS OF ^{239}Pu , ^{144}Ce AND ^{90}Sr CITRATE IN PRODUCING CHROMOSOME DAMAGE, BONE-RELATED TUMOURS, LIVER TUMOURS AND LIFE SHORTENING IN THE CHINESE HAMSTER.

Determination of the biological effectiveness for internally deposited α - and β -emitting radioactive materials using a number of endpoints is needed to make meaningful risk estimates for man. Late-occurring biological effects were studied in Chinese hamsters injected with ^{239}Pu , ^{144}Ce or ^{90}Sr citrate. Pu-239 is an α emitter which localizes in liver and bone, whereas ^{144}Ce and ^{90}Sr are β emitters which localize in liver and bone, and in bone, respectively. Biological alterations on the cellular, organ and organism level can be used in determining the relative effectiveness of α and β radiation in producing these endpoints. Significant life shortening was induced by all three radionuclides and was considered in evaluating time-related incidence of bone and liver neoplasms. Osteosarcomas were seen in animals injected with 1.0 μCi ^{90}Sr citrate and 0.002 μCi ^{239}Pu citrate/g body weight, while liver tumours including hemangiosarcomas, fibrosarcomas and hepatocellular neoplasms, were seen after injection of 0.25 μCi ^{144}Ce citrate and 0.002 μCi ^{239}Pu citrate/g body weight. Comparisons of bone and liver tumour incidences with cumulative radiation dose were made to derive quality factors for tumour production. In both cases, the derived factors (3.6 for bone tumours and 7.4 for liver tumours) were lower than the figure of 10 generally accepted for comparing α and β radiation. The factor derived from comparison of chromosome damage in liver cells was higher (15), indicating that other factors are involved between damage at the cellular level and final tumour production, and that direct correlations of genetic damage and tumorigenicity are hazardous.

INTRODUCTION

To experimentally determine the hazards of internally deposited radioactive materials, two basic complementary approaches can be used. The first is to test a single isotope in a variety of animal species, derive dose response relationships for each species and use basic biological similarities observed to predict what might occur in man. Another method involves relating the effects of several isotopes on varied biological endpoints in a single experimental animal species. In the latter case, the effectiveness of each isotope in producing comparable changes provides ratios which can be used for determining relative hazards. In the current research, we have used a single species, the Chinese hamster (*Cricetulus griseus*), injected with ^{144}Ce , ^{90}Sr ¹ or ^{239}Pu

¹ As used hereafter, ^{144}Ce and ^{90}Sr refer to these isotopes in equilibrium with their daughters, ^{144}Pr and ^{90}Y , respectively.

citrate, for evaluation of the relative effectiveness in production of several different endpoints. Dose-response relationships for chromosome damage, cancer induction and life shortening have been determined and effectiveness factors derived for the alpha emitter ^{239}Pu relative to the two beta emitters, ^{90}Sr and ^{144}Ce .

METHODS

Animals and radionuclides

Chinese hamsters, 100 days old, were injected with solutions of ^{144}Ce , ^{90}Sr or ^{239}Pu citrate using several different activity levels for each radionuclide. The ^{90}Sr and ^{144}Ce were injected intraperitoneally and the ^{239}Pu was injected intravenously. Control animals were injected with sodium citrate by comparable routes. Although there were several activity levels for each radionuclide, only one level of each was chosen for comparative evaluation in this paper. The activity levels of interest were those in which there was significant life-span shortening coupled with induction of neoplasms in the target organs of interest. In this way, comparisons could be made between survival and tumor production as well as with chromosome aberration frequency. The levels chosen for analysis were: 0.25 μCi ^{144}Ce /g body weight; 1.0 μCi ^{90}Sr /g body weight; and 0.002 μCi ^{239}Pu /g body weight.

Survival and dosimetry

In all three experiments, animals were either held for life-span observation or sacrificed at predetermined times after injection for evaluation of biological effects. Animals injected with 1.0 μCi ^{90}Sr or 0.002 μCi ^{239}Pu were allowed to live out their life-spans while those injected with 0.25 μCi ^{144}Ce were sacrificed at 700 days after injection. Evaluation of survival patterns was made using a life-table-type analysis [1]. Included in these data were animals that were sacrificed for a variety of analyses including radioanalytical and cytogenetic procedures. A parallel study was performed with each radionuclide to determine the retention and distribution patterns in various tissues and organs. This information was combined with the activity levels injected and the survival data to derive radiation doses to liver and bone. Doses were calculated assuming that 35% of the energy released by the decay of ^{144}Ce or ^{90}Sr was deposited in bone [2] and that 100% of the ^{239}Pu energy was deposited in either bone or liver. For ^{144}Ce , 65% of the energy was assumed to be deposited in liver [2]. It was further assumed that liver and bone weighed 1.5 and 3 g, respectively.

Cytogenetics

Analysis of chromosome aberration frequency was performed on liver cells of animals injected with ^{144}Ce and ^{239}Pu . Details of the methods and results have been reported [3]. Dose-response curves from these studies were used to derive the effectiveness factors reported here.

Pathology

All animals that died, were euthanized because they were moribund, or were sacrificed for observation of effects, were subjected to complete gross and microscopic evaluation. Tissue samples were routinely taken from lung, liver, spleen, kidney, adrenals, genital organs, muscle, bone, bone marrow and brain. Other tissues were taken if gross lesions were observed. Tissues were fixed in 10% neutral buffered formalin, embedded in paraffin, sectioned at 5 μm and stained routinely with hematoxylin and eosin. Special stains and autoradiographs were prepared from selected tissues as necessary. All tissues were evaluated for histopathological changes. Tumors were defined by site and histologic type. Cumulative incidence of neoplasms was evaluated by a modification of the Cutler-Ederer technique [4]. Only animals given complete pathological examinations were included in evaluation of tumor incidences.

RESULTS

The retention of ^{239}Pu in liver and bone of our animals had a long effective half-life because 85% of the initial activity was present 700 days after injection. The activity was distributed between liver (60%) and bone (40%). The ^{144}Ce citrate had a distribution similar to the ^{239}Pu and the effective half-life of the material in the whole body was close to the physical half-life of ^{144}Ce . More than 98% of the ^{90}Sr was deposited in bone and its whole-body retention could be adequately described by a three-component exponential $Y = 52.7 e^{-1.1t} + 36.7 e^{-0.083t} + 10.6 e^{-0.001t}$ where Y is the percent of the day zero count and t is time in days. Dose estimates to bone and liver were derived using injected activity, the fractional activity deposited in the organ of interest and the retention pattern in that organ. Because similar methods were used for all three radionuclides, cumulative dose as a function of time can be related to tumor incidence and to the observed frequency of chromosome damage. Table I shows cumulative doses to liver and bone for each radionuclide at the time of 50% cumulative tumor incidence in that organ.

Cumulative survival for the hamsters injected with the 3 radionuclides is shown in Figure 1. Injection of ^{90}Sr at 1.0 or 0.5 $\mu\text{Ci/g}$ body weight resulted

Table I

Comparisons of Survival, Dose and Tumor Incidences in Chinese Hamsters Injected with ^{90}Sr , ^{144}Ce and ^{239}Pu
Citrate

Tumor Site	Radio-Nuclide	Activity Injected ($\mu\text{Ci/g}$)	Total No. Animals Examined	Median Survival Time (DPI)*	Number Animals with Tumors	50% Cumulative Tumor Incidence (DPI)*	Cumulative Dose at 50% Tumor Incidence (rads)	Quality Factor for Tumor Induction
Bone	^{90}Sr	1.0	13	260	3	410	8 000	3.6
	^{239}Pu	0.002	52	540	13	610	2 200	
Liver	^{144}Ce	0.25	30	540	5	700	28 000	7.4
	^{239}Pu	0.002	52	540	12	630	3 800	

*Days post-injection

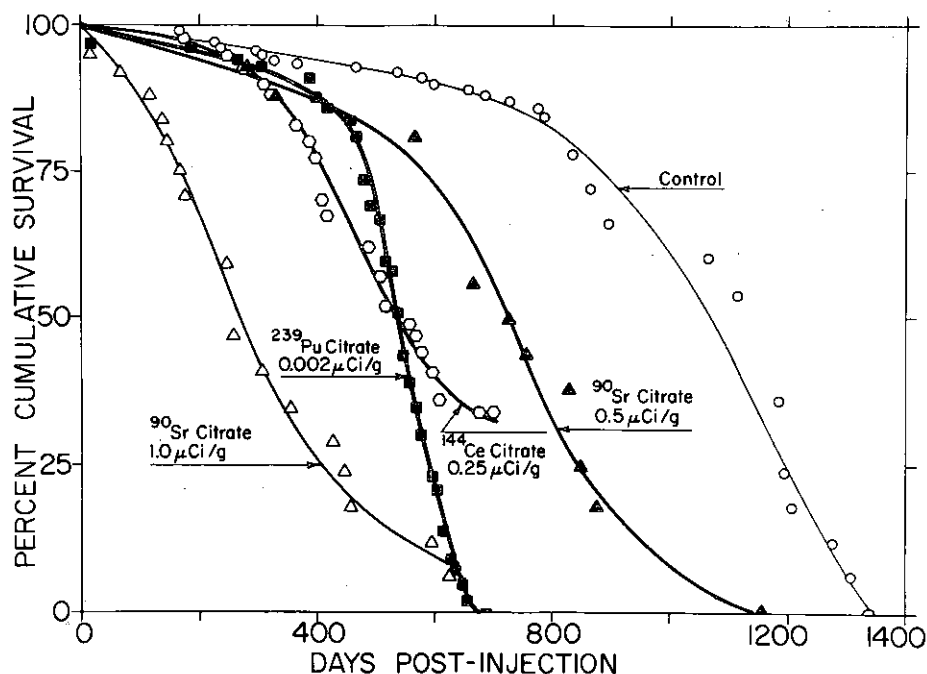


FIG.1. Cumulative survival in Chinese hamsters after injection of ^{90}Sr , ^{144}Ce or ^{239}Pu citrate. Significant lifespan shortening at the injected activity levels is shown for all three radionuclides.

in life shortening with median survival times of 260 and 700 days, respectively [5]. Injection of $0.25\text{ }\mu\text{Ci }^{144}\text{Ce}$ or $0.002\text{ }\mu\text{Ci }^{239}\text{Pu/g}$ body weight resulted in similar median survival times of 540 days. There were other differences, however, in that all animals included in our data that were injected with $1.0\text{ }\mu\text{Ci }^{90}\text{Sr/g}$ and $0.002\text{ }\mu\text{Ci }^{239}\text{Pu/g}$ died prior to 700 days whereas among those injected with $0.25\text{ }\mu\text{Ci }^{144}\text{Ce/g}$, a considerable number lived to be sacrificed at 700 days after injection. The use of life-table analysis adjusts for such differences, making more meaningful comparisons possible.

Deaths in all three groups of animals were, for the most part, related to radiation damage to the two primary target organs, skeleton and liver. Nine of 13 hamsters injected with $1.0\text{ }\mu\text{Ci }^{90}\text{Sr/g}$ body weight died between 60 and 449 days after injection because of bone marrow aplasias. Three died between 241 and 438 days with bone neoplasms classified as osteochondrosarcomas. In one animal, the cause of death was not specifically related to bone damage. Of 30 animals which died or were sacrificed after injection with $0.25\text{ }\mu\text{Ci }^{144}\text{Ce/g}$ body weight, five had developed liver neoplasms. Two of these were primary

fibrosarcomas arising in liver and one was a hepatic hemangiosarcoma. Two animals had neoplasms of hepatocellular origin which did not metastasize and were of questionable malignancy. These liver tumors were seen between 501 and 700 days after injection. The majority of the animals injected with ^{144}Ce also showed severe degenerative changes in the liver. Of 52 animals injected with $0.002 \mu\text{Ci } ^{239}\text{Pu/g}$ body weight, 12 developed liver neoplasms. Six of these were hemangiosarcomas and 6 were hepatocellular neoplasms. The tumors were morphologically similar to the corresponding tumors seen in animals injected with ^{144}Ce although one of the hepatocellular neoplasms was a frank carcinoma. Thirteen of the 52 animals also developed bone tumors (osteosarcomas or osteochondrosarcomas). The liver tumors were found between 395 and 686 days after injection and the bone tumors between 414 and 629 days. The majority of the animals injected with ^{239}Pu had severe hepatic degeneration.

A summary of the survival, tumor incidence and dose results outlined above is presented in Table I. The median survival times in each study are significantly shorter than the times to the 50% cumulative tumor incidence. The animals with ^{90}Sr -induced bone tumors died considerably earlier than those with ^{239}Pu -induced bone tumors, whereas the liver tumors in both ^{144}Ce - and ^{90}Sr -injected animals were seen over a similar time period. These differences in latent period for induction make comparisons of the effectiveness of the isotopes difficult. To make a meaningful comparison of liver or bone tumor incidence, it is essential to know the dose required for each isotope to produce the same level of biological change. This comparison was made by calculating the dose required to produce a 50% cumulative tumor incidence in either bone or liver. Equivalent tumor incidences were thus produced in bone after 8000 rads from ^{90}Sr or 2200 rads from ^{239}Pu , whereas it required 28 000 rads from ^{144}Ce and 3800 rads from ^{239}Pu to produce a 50% cumulative incidence of liver tumors. At lower levels of injected activity with ^{90}Sr ($0.5 \mu\text{Ci/g}$ body weight), there were no bone tumors found after a dose of 4000 rads over 800 days. In the animals injected with $0.25 \mu\text{Ci } ^{144}\text{Ce/g}$ body weight, a cumulative dose of 4000 rads to bone over a 700-day interval produced a single bone-related tumor at 608 days after exposure.

The frequency of chromosome aberrations after injection of ^{144}Ce or ^{239}Pu increased linearly with dose. The slopes of the dose-response lines were 3.1×10^{-4} and 4.8×10^{-3} aberrations/cell/rad for the ^{144}Ce and ^{239}Pu , respectively. The aberration type and distribution of aberrations in the cell population were similar for both isotopes even though the LET of the ^{239}Pu emissions was much higher than that for the ^{144}Ce .

DISCUSSION

A variety of biological endpoints from the level of the cell to the whole organism were evaluated in this study. Chromosome aberration frequency in the liver represents a cellular endpoint that can be measured at various times after injection. Cancer induction is also of prime interest in evaluation of health consequences, and life shortening is another measure of biological hazards associated with toxic materials. The radionuclides in question were selected because of their importance in the nuclear fuel cycle either as fuel or as waste products. Because ^{239}Pu localizes and is retained in liver and bone, it was important to use other isotopes which had similar retention patterns. Cerium-144 was selected because of its similar retention pattern and its importance as a fission product in a reactor after a period of sustained operation. Strontium-90 is another important fission product which concentrates and is retained in bone.

The long-term retention of ^{144}Ce and ^{239}Pu and the large fraction of the activity retained in the liver contrasts with retention seen in the rat [6] where ^{239}Pu and ^{144}Ce cleared rapidly and is more similar to the retention seen in dog [7] and man [8]. This suggests that the Chinese hamster may be a good model for studying long-term retention, removal of isotope and pathogenesis of radiation-induced disease especially as these relate to the liver.

When making comparisons of biological effects caused by toxic radioactive materials, one cannot simply use injected activity levels as is done with chemical toxins. Radiation dose to the critical tissues considers energy deposition but must also take into account distribution. Thus, comparisons of dose-effect relationships between ^{90}Sr and ^{239}Pu in bone must include the consideration that plutonium deposits on endosteal surfaces and strontium deposits throughout the bone volume. Because endosteal cells are generally considered to be the cell at risk for osteosarcoma induction, this becomes an important factor.

Using 50% cumulative tumor incidence as a biological endpoint, one can use the dose to the target organ at that time to compare relative effectiveness of tumor induction. In the case of bone, the cumulative dose at 50% tumor incidence was 2200 rads and 8000 rads for animals injected with ^{239}Pu and ^{90}Sr , respectively. This yielded a quality factor for bone tumor induction of 3.6; considerably less than the factor of 10 generally used by the ICRP to compare effectiveness of alpha and beta radiation in producing a given biological effect. Furthermore, if we add in the factor for the increased effectiveness of

the surface distribution of plutonium in producing bone cancer [9], the observed quality factor of 3.6 is much less than the suggested factor of 50.

The Chinese hamster appears more resistant to bone tumor induction than the mouse after injection with ^{239}Pu [10] or ^{90}Sr [11]. Mice injected with comparable levels of ^{239}Pu (0.0031 $\mu\text{Ci/g}$ body weight) developed 1.6 bone tumors/animal compared with 0.27 tumors/animal in our study. Mice injected with 1.0 μCi $^{90}\text{Sr/g}$ body weight reportedly developed 4.3 bone tumors/animal as compared with only 0.23 tumors/animal in Chinese hamsters. It has been calculated on the basis of retained body burden in mice that ^{239}Pu was 230 times more effective than ^{90}Sr in producing bone tumors [12]. If emission energy is taken into account, that factor is more like 50. A similar calculation for the dog, also taking energy into account, yields an effectiveness factor of 13. Both of these factors are higher than that derived here for the Chinese hamster. How the relative resistance of this species to bone tumor induction might affect these derived factors is not known.

On the other hand, the quality factor for the dose to liver at the 50% tumor incidence point (28 000 rads with ^{144}Ce and 3800 rads with ^{239}Pu) is 7.4 which is reasonably close to the predicted factor of 10. Using chromosome aberration frequency in liver cells as an endpoint, a quality factor of 15 can be derived for ^{239}Pu as compared with ^{144}Ce . This indicates that a direct relationship cannot necessarily be drawn between genetic or cellular damage and tumor production, and that other factors are probably involved between these two events.

In the experiments reported here, a relatively small number of experimental animal subjects have been used. Thus, the specific quality factors derived here are more of a conceptual value. They come relatively close to the predicted factor of 10 for alpha vs beta radiation, suggesting that it is unlikely that this figure is off by several orders of magnitude. In summary, the information derived from these experiments tends to agree with the generally accepted concepts used when comparing the relative toxicity of alpha- and beta-emitting radionuclides.

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DISCUSSION

H.H. ROSSI: You have given what you term the "quality factors" for 50% tumour incidence. Do you know what it would be for 10% or 1% incidence?

S.A. BENJAMIN: We do not have meaningful data on the "quality factors" at either high or low cumulative tumour incidences. In the case of the groups discussed, the animals generally died with neoplasms within a restricted and finite time span (about 200 days), so that doses calculated did not vary greatly at the time of 10, 50 or 90% cumulative tumour incidence. The 50% incidence value was felt to be most reasonable for

such a comparison. At lower injected ^{239}Pu activity levels we may well be able to calculate these values for 1% or 10% cumulative tumour incidence. These studies are under way but we do not yet have enough data on these animals.

C. W. MAYES: Speaking on the present occasion on behalf of the US National Council on Radiation Protection, I should like to point out that the quality factor for α emitters (10) and the distribution factor for plutonium in bone (5) were intended for the low doses of greatest concern for radiation protection, not the very high doses which you have compared, and which Marvin Goldman refers to as "incandescent". Miriam Finkel and others have shown that the dose-response for the β -emitter ^{90}Sr is highly sigmoid while that for α emitters often tends to be linear, so that while the toxicity of ^{239}Pu is much higher than that of ^{90}Sr at low doses, their toxicity ratio decreases at higher doses.

S. A. BENJAMIN: Your point is well taken. It is important to note that in the studies from which we have taken these data there are groups which were injected with significantly lower activity levels. The ^{90}Sr and ^{144}Ce studies include several groups in each of which the injected activity levels extend down to as low as one-seventh of the levels discussed. The ^{239}Pu study includes groups with 10, 100 and 1000 times lower injected activity levels than those being evaluated. To date (about 700 days post injection) virtually no effects have been seen in the animals with lower ^{239}Pu activity levels. Comparisons between the lower-level groups for all the radionuclides will be made as data become available.

K. H. CLIFTON: I was struck by the difference in lifespan between your control animals and those of D. M. Smith and co-workers at Los Alamos (paper SM-202/410). What effect do you think this might have on the respective results?

S. A. BENJAMIN: It should be made clear that we are dealing with two completely different species. Dr. Smith reported on the use of the Syrian hamster (Mesocricetus auratus), while our work has been on the Chinese hamster (Cricetulus griseus). They are animals with different lifespans, as you have noted. The Chinese hamster is a relatively long-lived rodent. Many people confuse these two species, and the distinction should therefore be emphasized.

GROSS DISTRIBUTION OF ^{241}Am IN A MAN SEVEN YEARS AFTER INHALATION*

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Abstract

GROSS DISTRIBUTION OF ^{241}Am IN A MAN SEVEN YEARS AFTER INHALATION.

The gross distribution of ^{241}Am remaining in a man seven years after exposure to airborne americium particles has been determined by several techniques. Measurements were made of the radiation with NaI(Tl) crystals, one above and one below the supine subject at seven equally spaced points along the long axis. The patterns obtained indicated skeletal deposition in addition to material in the thorax. More detail was obtained from a longitudinal profile scan made at 10-cm intervals with a slit-collimated detector. A general labelling of the skeleton consistent with bone surface deposition was observed, together with a pronounced increased concentration in the thorax. A transverse profile scan was made at the point which had yielded the highest counting rate in the longitudinal scan. Unequivocal evidence for a lung burden was observed in the asymmetry of this scan. The presence of the long-term lung burden was further confirmed by measurements made over the chest with a gas-filled proportional counter. The ratio of primary quanta in the 60-keV peak to those which had been forward scattered indicated that the radiation had passed through approximately 5 cm of soft-tissue equivalent absorber. Since estimates of the subject's chest wall thickness were only about 2.5 cm, the major part of the radiation was coming from deeper in the chest, below the level of the rib cage. Therefore the material in the chest was primarily in the lungs and not in the bones of the thoracic cage. The proportional counter also showed that there was little contamination in the liver and spleen. This was expected since the subject had undergone extensive chelation therapy with DTPA at another centre. The whole-body content of ^{241}Am was estimated to be $1.00 \pm 0.03 \mu\text{Ci}$, with about 25% of this amount in the lungs. Thus a sizable fraction of the material retained is still in the lungs after seven years.

1. INTRODUCTION

In 1967, four individuals, who had been exposed in an industrial plant to airborne particles of americium over a period of several months in late 1966, were investigated at Argonne National Laboratory for possible internal contamination. Measurements of the 60-keV γ ray with a NaI(Tl) crystal showed that one man had a burden at least fifty times that of the others. A series of measurements was then made to determine the apparent distribution of the isotope within his body. The results indicated wide-spread deposition in the skeleton in addition to material remaining in the lungs. The relative uptakes of ^{241}Am in different parts of the skeleton were consistent with the assumption that americium was deposited on bone surfaces and therefore was concentrated more in trabecular bone than in cortical bone [1]. Since that time, this subject has undergone extensive chelation therapy with DTPA¹ at a hospital [2].

* Work performed under the auspices of the US Energy Research and Development Administration.

¹ Diethylenetriaminepentaacetic acid.

The subject visited the Center for Human Radiobiology in 1973 and new measurements were made in order to determine the gross distribution of radioactivity in his body seven years after exposure.

2. γ -RAY MEASUREMENTS

2.1. Seven-Position Scans

Measurements of the 60-keV γ ray were made along the body of the supine subject with a modified "seven-position-scan" technique, originally developed by Miller [3]. Two 29.2-cm diameter by 10.2-cm thick NaI(Tl) detectors were used, one at 30 cm above the bed and the other at 10 cm below the bed. The fourth position was at the midpoint of the subject's height, and the interval between successive positions was 15% of the subject's height.

The results of these measurements indicated the general longitudinal distribution of ^{241}Am in the body and are shown in Figure 1. The peak at position 2 arises from activity in the vertebrae, ribs and thorax. The higher counting rates from the upper crystal at positions 5 and 6 indicate activity in the bones of the legs, and especially in the knees, since more soft tissue shields the lower crystal than the upper, even though the legs are much closer to the lower crystal than to the upper.

These scans indicate that much of the activity in the thorax is actually in the lungs for the following reasons: 1) a higher counting rate is observed from the back than from the front; 2) the maximum counting rate appears to be nearer the vertex when measured from the front than from the back; and 3) a broader peak in the counting rates is observed from the back than from the front. All these characteristics can be predicted from a consideration of the size, shape and positioning of the lungs in the thoracic cavity.

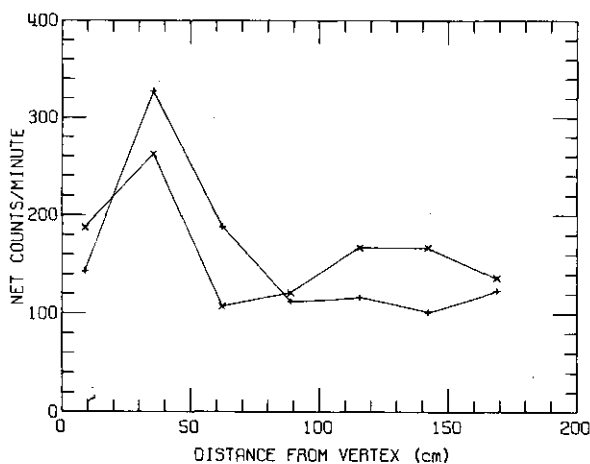


FIG.1. Seven-position scans of the subject. x: counting rate from the upper crystal; +: counting rate from the lower crystal.

2.2. Profile Scans

In order to determine more exactly the distribution of radioactivity in the subject's body, a longitudinal profile scan was made. A lead collimator with a 2.54-cm-wide slit transverse to the long axis of the body was placed over the lower detector and measurements were taken at 10-cm intervals. The interval was decreased to 5 cm in regions where the response from ^{241}Am differed markedly from one position to the next. Figure 2 shows the profile obtained. Peak counting rates occur in the regions of the skull, chest, pelvis, knees and feet, consistent with the 1967 results, which indicated labelling of the entire skeleton but especially trabecular bone. The large peak from the chest is due to material both in bone and in the lungs, and the asymmetry of this peak at 50 cm from the vertex may be due to material in the liver and spleen, or in the rib cage and vertebrae. The aperture was then turned through 90° so as to be parallel to the long axis of the body, and a transverse profile scan was made of the chest at 40 cm from the vertex, the position which yielded the highest counting rate in the longitudinal scan. The transverse scan is shown in Figure 3. The asymmetry of this scan indicates that the response came primarily from material in the lungs rather than in the bone, since the right lung is larger than the left. Similar patterns of activity have been observed in several volunteers who inhaled a radioactive aerosol as part of an intercalibration experiment for the detection of plutonium in vivo [4].

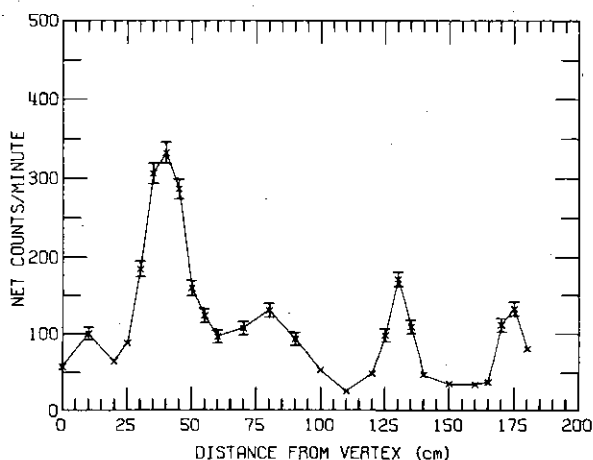


FIG.2. Longitudinal profile scan of the subject. These measurements were made with a 2.54-cm-wide aperture on the lower crystal.

2.3. Whole-Body Content

The subject's radioactivity was measured with a 29.2-cm diameter by 1.27-cm thick NaI(Tl) crystal while he lay on a curved bed of 1.5-m radius. Measurements were made both with the subject facing the detector and with his back to the detector, which was mounted 1.37 m above the bed. The efficiency of detection of the 60-keV γ ray in this

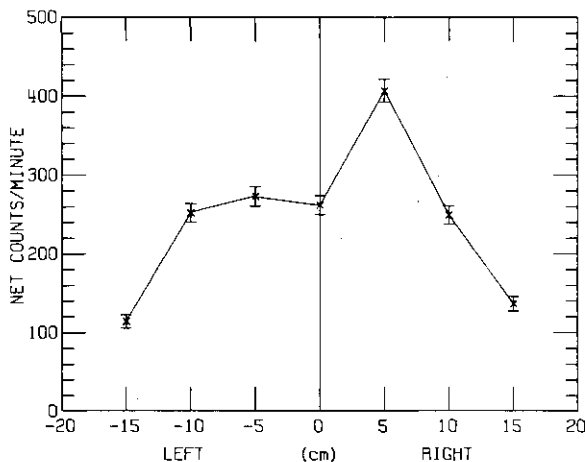


FIG. 3. Transverse profile scan of the subject at 40 cm from the vertex. The positions are left and right of the median line of the body.

configuration varied along the length of the bed. A correction was made for this by weighting the efficiency observed at each of several positions along the curved bed with the relative radioactivity of the subject at that position, obtained from the seven-position scan.

Transmission measurements through tissue-equivalent material established the mass attenuation coefficient for the 60-keV γ ray to be $0.130 \text{ cm}^2/\text{g}$. Because the NaI(Tl) crystals are unable to resolve scattered radiation from primary quanta in the 60-keV peak, a correction for forward scattering was necessary. This correction amounted to 28%. With the use of these values and of transmission data for the subject (measured at the upper back, lower back and knee), the effective average thickness of the body was determined to be 12.4 cm.

With the assumption that the effective center of the radioactivity was at the midplane of the body, the whole-body burden of ^{241}Am was calculated to be $1.0 \pm 0.03 \mu\text{Ci}$. This value, when added to the total amount of ^{241}Am excreted under chelation therapy [5], indicates an initial deposition of $2.2 \mu\text{Ci}$, some 20% higher than was estimated in 1967 [1].

3. MEASUREMENTS AT HIGHER RESOLUTION

In order to estimate directly the remaining lung burden of this subject, measurements were made with an 18-cm-diameter xenon-filled proportional counter placed over midsternum of the supine subject. This counter has much higher resolution than the NaI(Tl) crystal and has the ability to resolve the ^{237}Np LX rays, as well as scattered γ rays.

3.1. Determination of Effective Source Depth

In order to determine the amount of ^{241}Am present in the lungs of this subject, the amount of absorber interposed between the source and

detector had to be known. This quantity (the effective soft-tissue thickness) was estimated from the equation suggested by Rundo et al. [6]. (However, their empirical equation was derived for 20-keV photons, and may not be valid at 60 keV.) For this subject the effective soft-tissue thickness, which allows for the lower density of lung tissue, was 5.17 ± 0.73 cm. Normally, a calibration factor is then obtained by layering tissue-equivalent absorber over a source of ^{241}Am to obtain a broad-beam attenuation curve, and a calibration factor (cpm/ $\mu\text{Ci } ^{241}\text{Am}$) is interpolated from this curve.

However, this procedure could not be applied directly to this subject because of the possibility of an unknown fraction of the total chest burden lying in the ribs, with the rest lying in the lungs. Consequently, an alternative empirical method of determining the amount of absorber through which the ^{241}Am radiations had passed was developed [7].

Briefly, the method consists of measuring the ratio of scattered quanta to primary quanta from the 60-keV transition. The region from 35 to 45 keV consists entirely of scattered radiation, and the ratio of counts in this region to those in the 60-keV peak was found to be a linear function of the amount of tissue-equivalent absorber covering a ^{241}Am source.

The proportional counter spectrum obtained from the chest of this subject is shown in Figure 4. The peak at 26.3 keV is due to a γ ray from decay of ^{241}Am and the conversion X rays are at 13.9, 17.8 and 20.8 keV. The peak at 30 keV results from the escape of a 30-keV xenon X ray following complete ionization of an atom of the counting gas by a 60-keV γ ray.

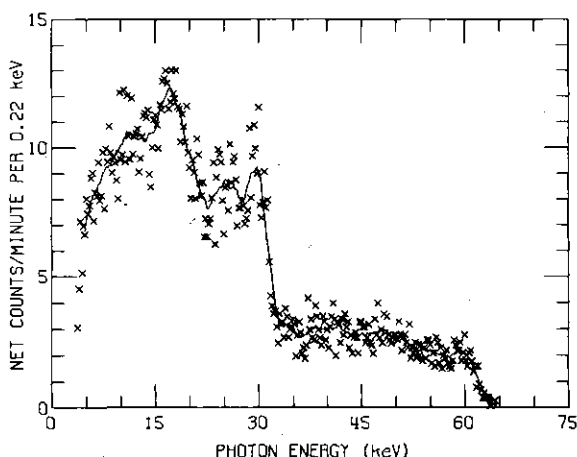


FIG.4. The spectrum of ^{241}Am obtained with the proportional counter centred over mid-sternum of the subject. The solid line was obtained by smoothing the experimental points over eleven-channel intervals; this results in considerable loss of resolution in the region below 35 keV.

TABLE I. Effective depth of ^{241}Am deposited in various regions of the body

Location	Skull	Thorax	Liver	Left side	Knees
Effective depth, cm ($\pm 10\%$)	0.75	4.86	11.8	12.3	2.47

In the spectrum of Figure 4, the ratio of scattered counts to peak counts indicates an absorber thickness of 4.86 ± 0.49 cm. This figure agrees quite well with the estimated effective soft-tissue thickness of 5.17 ± 0.73 cm for radiation in the 20-keV region, confirming that the burden lies primarily in the lungs. A small fraction of the total amount present lying in the rib cage cannot be ruled out because of the rather large limits of precision of these numbers. For instance, a combination of 80% of the total amount of ^{241}Am present under 5.25 cm absorber and 20% of the total under 2.5 cm absorber gives a scattered-to-peak ratio lying within the lower limit of that obtained from the subject. However, the proportional counter, when placed over midsternum, directly views only a small fraction of the bone in the rib cage (except for the sternum itself). Consequently it is unlikely that ^{241}Am in the rib cage is contributing an appreciable fraction of the observed response.

The same technique was used to estimate the effective depth of ^{241}Am deposited in other regions of the body. The results are shown in Table I and all are consistent with what would be expected from anatomical considerations.

The counting rate observed when the detector was positioned over the liver was nearly equal to that observed over the left side. The absence of ^{241}Am from the liver was no doubt due to the chelation therapy.

3.2. Calibration Factor for ^{241}Am in the Lungs

Since the empirical method of determining the depth of ^{241}Am deposited in the thorax gave a value which agreed well with the estimated effective soft-tissue thickness, a calibration factor was obtained as follows. A point source of ^{241}Am was placed at a distance from the counter equal to the half-thickness of the subject's thorax. Tissue-equivalent absorber was placed over the source to obtain a broad-beam attenuation curve, and a calibration factor was interpolated from this curve for 4.86 cm of absorber. Application of this factor to the counting rate measured over the subject's chest gave a value of $0.23 \pm 0.03 \mu\text{Ci } ^{241}\text{Am}$ for the lung content. The same value was obtained from counting either the X-rays or the 60-keV γ rays. In the case of the X-ray region, however, it was evident (see Figure 4) that scattered radiation was making a sizable contribution to the counting rate observed. This contribution was probably offset by the total absorption of X-rays in the rib cage. However, an exact method of determining the contribution of scattered radiation to this region remains to be developed.

Since americium deposited in the rib cage and sternum lies much closer to the counter than that distributed throughout the lung (and possibly thoracic lymph nodes), a sizable fraction of the thoracic content present in the rib cage would result in an estimated total thoracic content much lower than that given above. Such a lower content would be inconsistent with the results of the profile scans presented in Section 2.2.

4. CONCLUSIONS

A strong correlation between the distribution of activity measured in the longitudinal profile scan and the available bone surface areas may be obtained if two assumptions are made: first, the lungs contain some 25% of the total body burden, and second, the calibration factor for ^{241}Am does not vary markedly from one position of the profile scan to another. The distributions of counting rate and surface area are presented in Table II. The correlation between the two distributions is +0.94, which is significant at the 99% level.

TABLE II. Correlation of observed counts in the profile scan with bone surface area ($\rho = +0.94$)

Region	Portions of skeleton	% total surface area ^a	% total counts (less lung content)
I	Skull 4 cervical vert.	12	14
II	3 cervical vert. Thoracic vert. Chest cage Humeri	26	23
III	Lumbar vert. Pelvis Proximal femurs Radii Ulnae Hands	33	26
IV	Distal femurs Proximal tibiae Proximal fibulae	17	22
V	Distal tibiae Distal fibulae Feet	12	15

^a Derived from the distribution of cortical and trabecular bone by mass for reference man (ICRP Publication 23 (1975) p. 67).

There are four major conclusions which may be drawn from our measurements on this subject:

1. Chelation therapy has resulted in the removal of more than one-half the initial body burden.
2. There is little activity in the liver—presumably due to removal of ^{241}Am by the chelation therapy.
3. Some 10% of the initial body burden remains in the lungs.
4. Distribution of the remaining activity is consistent with deposition on bone surfaces.

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DISCUSSION

Miriam FINKEL (Co-chairman): Have there been any roentgenographic changes in the skeleton or lung or any blood changes in the individual concerned?

R.E. TOOHEY: All our patients are given complete physical examinations and a set of radiographs of the entire skeleton is made. So far no skeletal or blood changes have been noted. A "suspicious shadow" was observed in one lung by our radiologist, but a more detailed investigation by the patient's personal physician showed that there was no cause for concern.

R.O. McCLELLAN: Several years ago Dr. T. Mercer published a paper expounding a theory of particle dissolution, in which the dissolution rate for particles was related to their surface area. Our Institute and other laboratories have reported differences in dissolution rate for various transuranics from a very low rate for $^{239}\text{PuO}_2$ to successively higher values for $^{238}\text{PuO}_2$, $^{241}\text{AmO}_2$ and $^{244}\text{CmO}_{1.7}$. Mercer's theory and our own data would suggest that you should see a changing rate of loss of radioactivity from lung which is related to the changing particle size distribution resulting from the influence of dissolution. Have you had the opportunity to observe such a change?

R.E. TOOHEY: No, we have not. The 1967 measurements gave about $0.5 \mu\text{Ci}$ in the lungs and the more recent measurements $0.23 \mu\text{Ci}$. So we have only two data points, and of course we cannot determine a change in the clearance rate from the lungs just with those. However, we shall continue to follow this case, so perhaps in the future we shall be able to answer your question.

EFFECT OF ^{241}Am ON BONE STRUCTURE ACCORDING TO ITS MICRODISTRIBUTION

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Abstract

EFFECT OF ^{241}Am ON BONE STRUCTURE ACCORDING TO ITS MICRODISTRIBUTION.

Female rats, eight weeks old, were injected intravenously with monomeric $^{241}\text{Am}(\text{III})$ -citrate ($30 \mu\text{Ci/kg}$) and sacrificed after 7, 28 and 56 days. Half of the animals received once weekly an intraperitoneal injection of Ca-DTPA ($30 \mu\text{mol/kg}$), the first dose being administered 1.5 h after ^{241}Am . From the distal end of the femurs, 20- μm -thick longitudinal and sagittal sections were prepared. Both dose-rate distributions and morphometric parameters were determined by an electronic image analyser by scanning cellulose nitrate α detectors or microradiographs. Initially after injection, ^{241}Am is heavily deposited on the epiphyseal and metaphyseal sides of the cartilage plate as well as near the articular cartilage, whereas the labelling of the periosteal and endosteal surfaces in cortical bone and of all trabecular surfaces is less pronounced. Seven days after injection the maximum dose rate in the metaphyseal band amounts to $\sim 120 \text{ rad/d}$ and increases to 170 rad/d at 56 days. Deformations of the trabecular structure are presented quantitatively as deviations in the frequency function of chord lengths over trabeculae or marrow spaces. The endosteal surface area is lower in ^{241}Am animals than in controls and the complexity of the bone structure is reduced. In control animals the surface/volume ratio varies between 36 mm^{-1} and 64 mm^{-1} . The mean trabecular width ranges between $40 \mu\text{m}$ and $100 \mu\text{m}$ and the mean width of the marrow spaces range between $90 \mu\text{m}$ and $210 \mu\text{m}$. As a result of the locally high activity, the mechanism of bone resorption is strongly impaired in the metaphysis, manifesting as abnormal trabeculation. There is no pronounced diminution of DTPA efficacy over the period of observation. Ca-DTPA treatment lowers the dose rates in the metaphysis and as a consequence the inhibition of bone resorption seems to be partially suspended.

1. INTRODUCTION

^{241}Am is a bone-seeking radionuclide with high affinity to endosteal surfaces. This implies an inhomogenous deposition in the skeleton and locally high dose rates for small amounts of the incorporated nuclide. As the populations of osteogenic cells forming and remodelling bone tissue are preponderantly affected by the radiation energy dissipated in tissue, morphometric changes may be anticipated. Such morphometric alterations and their relationship to the microdistribution of ^{241}Am are the concern of this paper.

2. METHODS

For this experiment, eight-week-old rats of the Heiligenberg strain were used. $30 \mu\text{Ci/kg}$ Am(III) was injected i.v. as monomeric citrate complex. One group of animals was treated with weekly i.p. injections of Ca-DTPA¹ ($30 \mu\text{mol/kg}$), the first dose being given 1.5 hours after injection of the nuclide. Another group comprised animals injected with ^{241}Am and

¹ DTPA = Diethylenetriaminepentaacetic acid.

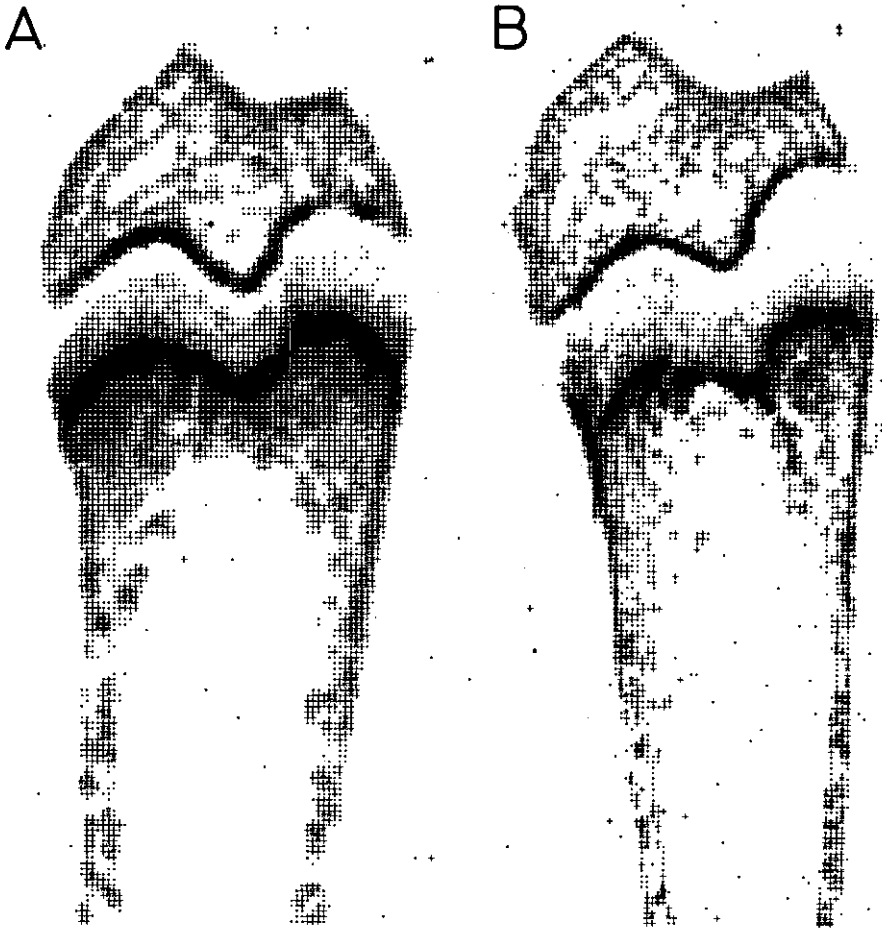


FIG.1. Scan of rat femur (distal end). $30 \mu\text{Ci/kg } ^{241}\text{Am(III)-citrate}$.

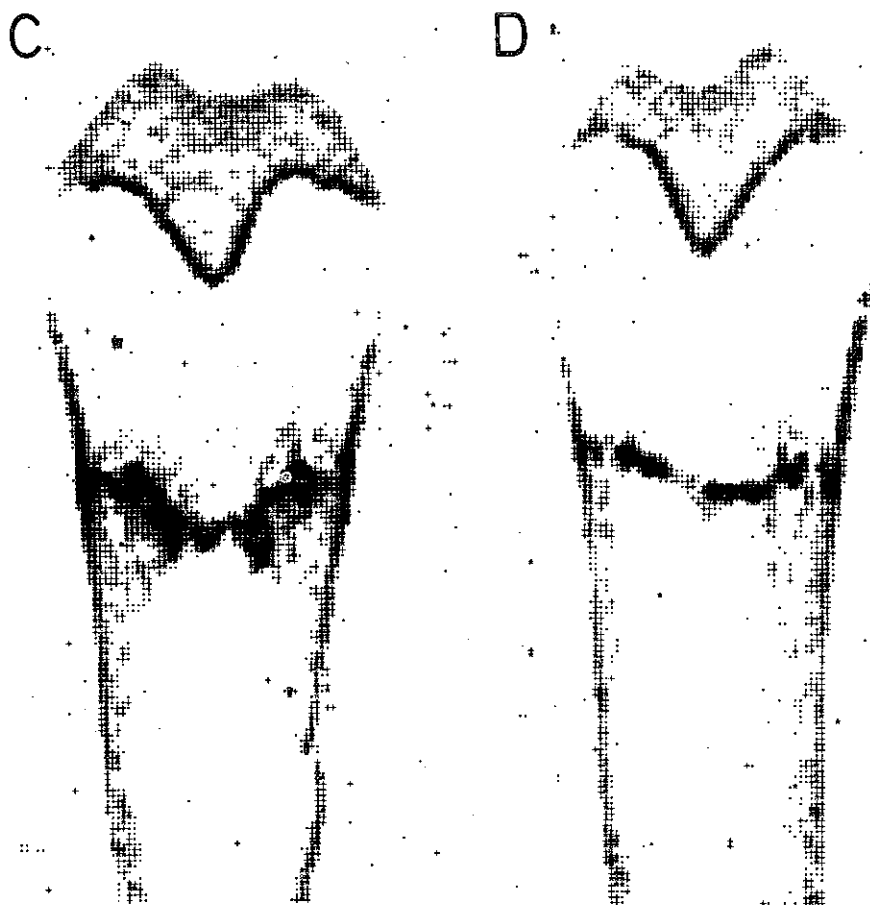
(A) 7 days; (B) 28 days; (C) and (D) 56 days after injection. (D) Ca-DTPA treated with weekly doses of $30 \mu\text{mol/kg}$.

Plot code (rad/d): blank <5; • 5-10; + 10-20; * 20-40; *+ 40-80; *ω >80.

was not subject to DTPA treatment. A third group of animals which received no ^{241}Am was kept as control. The animals were sacrificed at 7, 28 and 56 days, respectively. When embedded in methylacrylate, $20\text{-}\mu\text{m}$ sagittal and longitudinal sections were prepared from the undecalcified distal half of the femur.

Two adjacent sections were always taken, one for recording the ^{241}Am distribution by exposure of cellulose nitrate detectors [1], the other for microradiography with soft X rays (7.5 kVp) on Kodak high-resolution plates. Both the etched (30°C , 6.5 N NaOH , 3 hours) cellulose nitrate detectors and the microradiographs were evaluated by means of the electronic image analyser Quantimet 720.² The track density distribution on the α detectors

² IMANCO, United Kingdom.



was determined in square scanning fields of $80\text{ }\mu\text{m} \times 80\text{ }\mu\text{m}$ by measuring the area covered by α tracks [2]. The scan and morphometric data were processed by an on-line connected WANG 720 computer system [1, 3].

3. RESULTS AND DISCUSSION

Figure 1 displays the computer-generated plots of six dose-rate levels obtained by scanning the cellulose nitrate detector samples. The gross distribution pattern in longitudinal sections shows that there is a rapid initial deposition of ²⁴¹Am in the zone of endochondral ossification in the metaphysis and a comparable but somewhat lower concentration at the epiphyseal side of the cartilage plate (Fig.1(A)). Other regions of the metaphysis and epiphysis show a relatively homogeneous labelling due to the fact that bone turnover and remodelling in young rats are high, so that by the processes

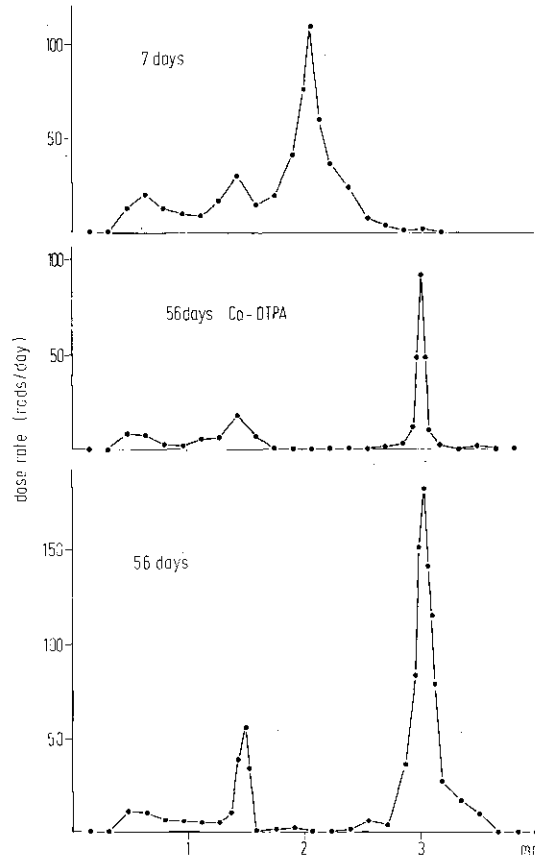


FIG.2. Dose-rate profiles along the bone axis in a strip 800 μm wide. The three peaks in each panel correspond, from left to right, to the deposits on the articular cartilage, epiphyseal cartilage and metaphyseal band.

of burial and recirculation ^{241}Am is not only deposited on the bone surfaces but also throughout the volume of calcified tissue. The formation of new bone and the consequent longitudinal growth bring about a shift of the metaphyseal band towards the diaphysis (Fig.1(A,B)) accompanied by a reduction of the ^{241}Am concentration round the metaphyseal band. The deposit on the epiphyseal side of the cartilage plate seems to be stable during the time of observation.

Upon comparison of Fig.1(C) and (D), a significant effect of DTPA may be verified, especially in a zone around the metaphyseal band. But there is no pronounced diminution of the ^{241}Am deposition at the epiphyseal side of the cartilage plate and throughout the epiphysis. This may also be demonstrated by Fig.2, which represents the dose-rate profiles in a strip 800 μm wide oriented along the bone axis in the middle of the sections. Between 7 and 56 days, the dose-rate maximum corresponding to the metaphyseal band becomes sharper and increases from 120 rad/d to 170 rad/d. The area under the metaphyseal peak of DTPA-treated animals is distinctly lower than for the untreated controls.

The deposition of ^{241}Am has some effect on bone structure, as can be seen from the microradiographs inserted in Fig.3. In control animals, the trabecular structure in the epiphysis is relatively ordered and regular, whereas in ^{241}Am animals the trabeculae look more irregular and fragmented (Fig.3(A)). The frequency distribution of the trabecular width measured in the direction indicated by the arrows gives a quantitative impression of the structural differences between the two sections. The frequency functions for the dimensions of the marrow spaces, however, show no significant differences in the epiphysis (Fig.3(C)), which is in contrast to the region of the epiphyseal plate where this function is shifted towards lower values in ^{241}Am animals.

The dependence of the perimeter L of the bone-soft-tissue phase boundaries (a measure of the endosteal surface) on the position of the sagittal sections can be inferred from Fig.4(A). The area of the endosteal surface, which increases in control animals during the period of observation, is distinctly reduced in all parts of the distal femur of ^{241}Am animals after 56 days. This decrease of the perimeter is caused by a loss of structural details and a coarsening of the trabeculae; this is corroborated by the fact that the factor $^3C = L^2/4\pi A$, being a measure of the complexity of the structure, is generally lower for ^{241}Am animals than for controls (Fig.4(B)). The surface/volume ratio S_V was measured because this quantity is considered to be a decisive parameter for evaluating relative risks of radiation effects from surface-seeking radionuclides in different species and because S_V determines the number of irradiated cells. To facilitate comparison with other investigations, two curves are drawn in Fig.4(C), one related to total bone volume (S_{VT}), the other to hard-tissue volume alone (S_{VB}). The latter parameter varies between 36 mm^{-1} and 64 mm^{-1} and is a little higher than for the vertebra of the mouse ($25 - 35 \text{ mm}^{-1}$) [4] and of the beagle [5] and distinctly higher than for the iliac crest (17 mm^{-1}) [6] and the femur head (9.6 mm^{-1}) [4] of man.

A qualitative comparison of the bone structure between inactive, ^{241}Am - and DTPA-treated animals (Fig.5) reveals some alterations manifesting in particular as calcified tissue remnants in the metaphysis coinciding with the metaphyseal band (see profile of the ^{241}Am deposition on the far right of Fig.5). This zone of abnormal trabeculation is absent in animals treated with DTPA (Fig.5(B)). Just above the location of the metaphyseal deposition peak, the trabeculae are large and coarse, indicating an impaired bone resorption, while bone apposition is obviously still active. Even near the epiphyseal cartilage plate where the dose rates from recirculating and redeposited ^{241}Am are very low, this effect is perceptible in ^{241}Am animals (Fig.5(C)). Autoradiographic studies show that the efficacy of DTPA, which is continuing in growing rats during the time of observation, is caused by inhibition of the redeposition of released ^{241}Am . Thus the labelling of newly formed bone above the metaphyseal band is absent in treated animals and consequently no pathological manifestations of bone structure are detectable in that portion of the femur (Fig.5(B)). The mean trabecular width varies between about $100 \mu\text{m}$ in the epiphysis and $40 \mu\text{m}$ in the central region of the epiphyseal plate where the delicate structures of the primary spongiosa contribute to the lower mean (Fig.6). Thus, trabecular dimensions in the

³. The factor 4π is for normalization, so that for a single circle $C = 1$.

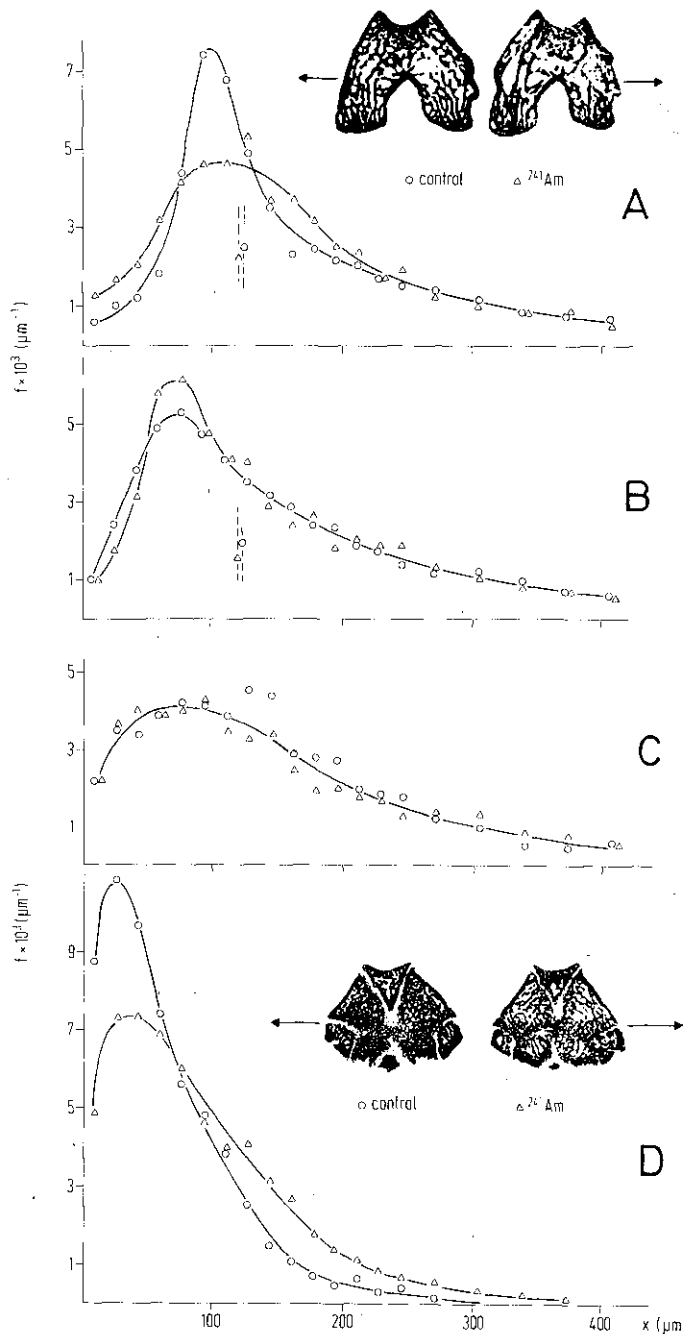


FIG.3. Frequency function of the trabecular width (A) (B) and the width of marrow spaces (C) (D) measured along the direction as indicated by the arrows, eight weeks after injection of ^{241}Am . (A) (C) epiphysis; (B) (D) epiphyseal plate.

The dashed lines correspond to the position of the mean.

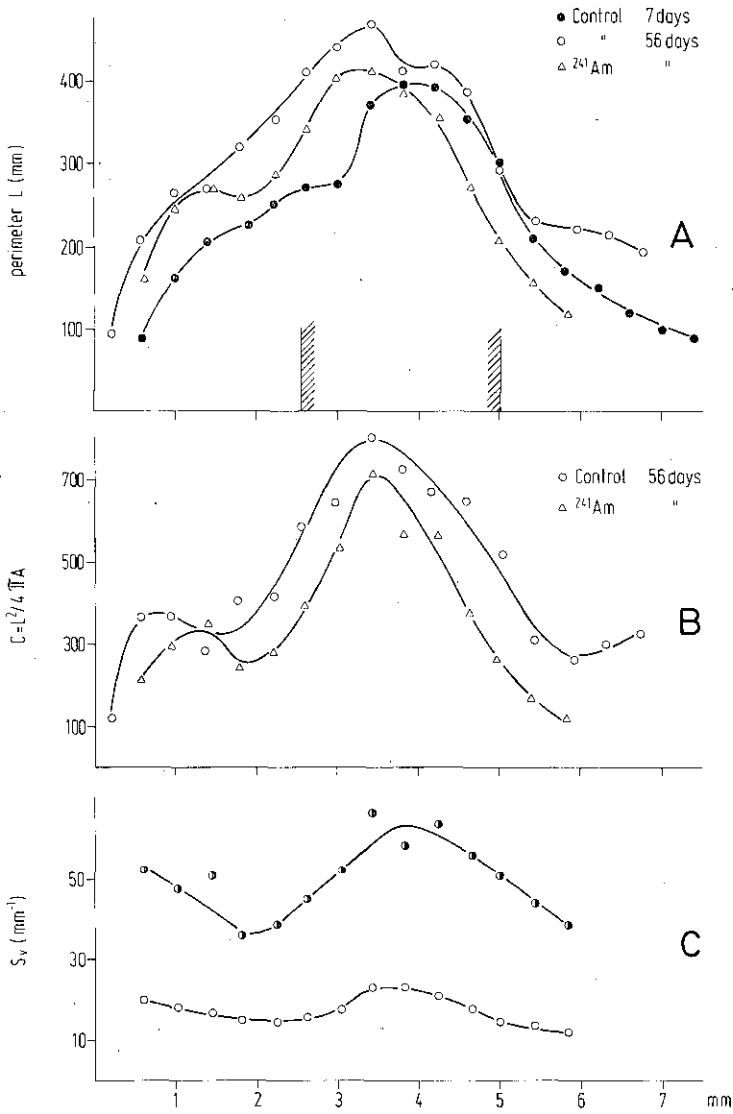


FIG.4. A: Perimeter L of bone-marrow interphase boundaries as a function of distance along the bone axis, measured in sagittal sections. The region of the epiphyseal plate is indicated by vertical lines and hatching.
 B: Quantity $C = L^2 / 4\pi A$, which is a measure of the complexity of bone structure. A = total area of bone tissue in the section.
 C: Surface/volume ratio S_V referred to total bone volume S_{VT} (○) and bone tissue volume S_{VB} (●). Control 56 days.

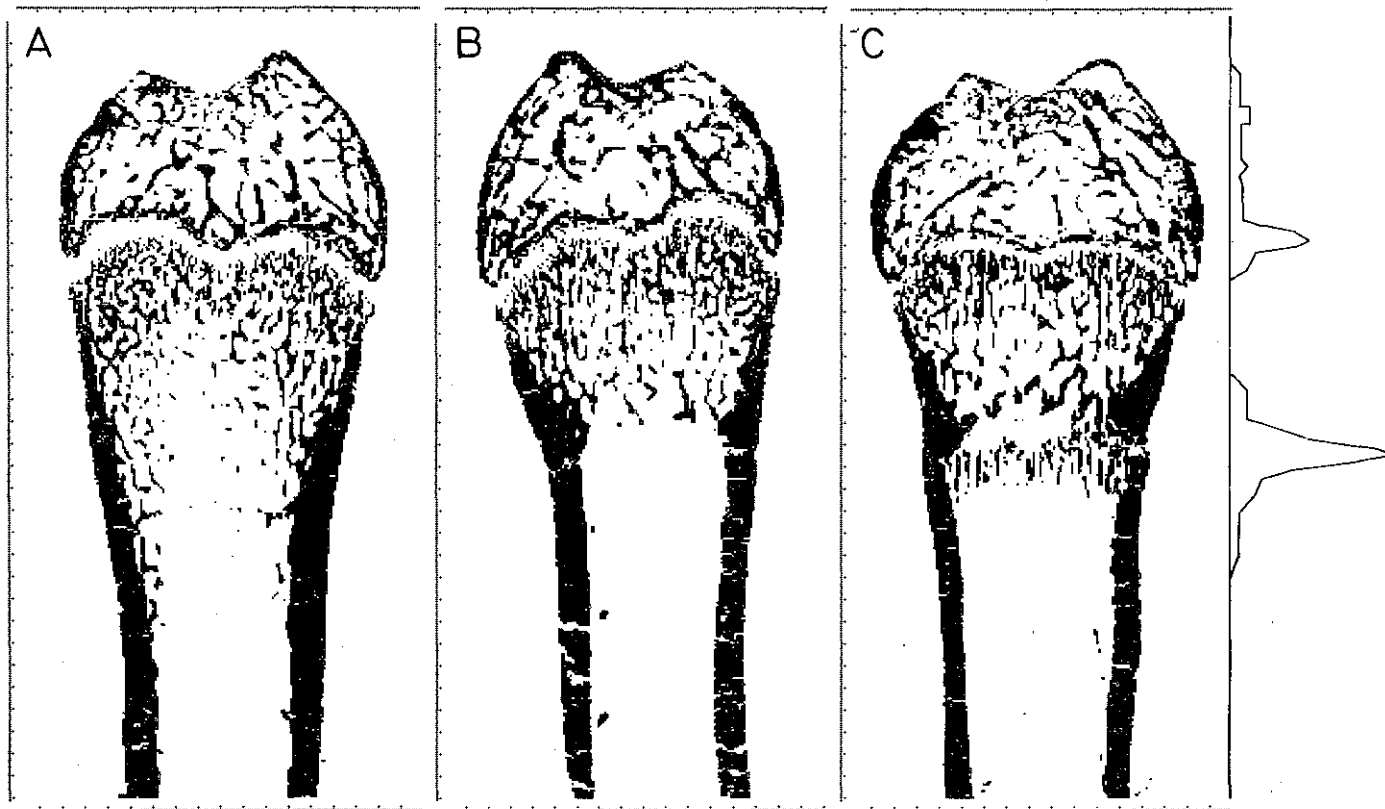


FIG.5. Distal half of the femur. (A) control; (B) ^{241}Am and treated with Ca-DTPA; (C) ^{241}Am untreated. Eight weeks after injection of ^{241}Am . The profile on the far right indicates the deposition of ^{241}Am (relative scale).

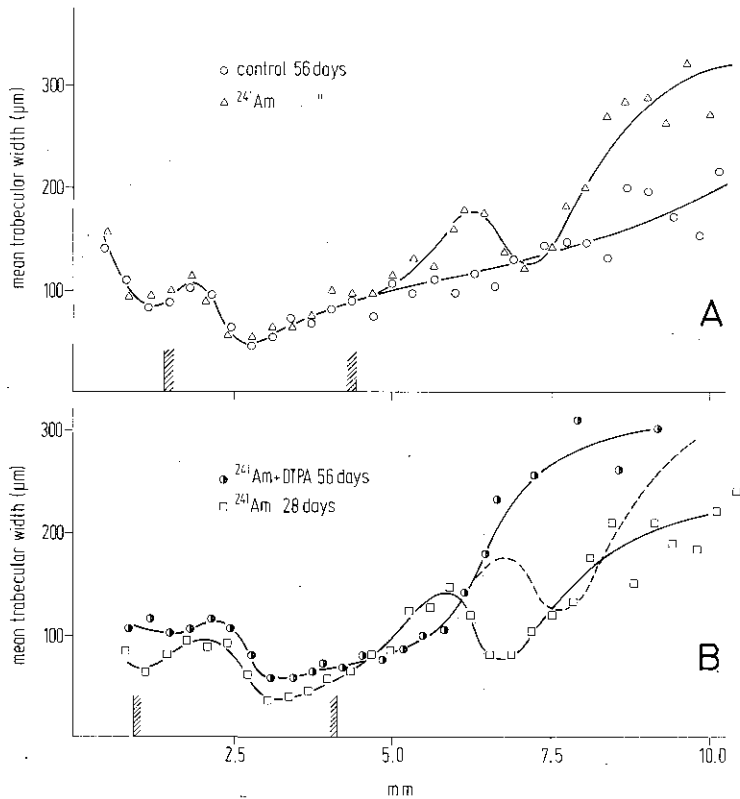


FIG.6. Mean trabecular width measured in a direction perpendicular to the bone axis. The region of the epiphyseal plate is indicated by vertical lines and hatching. The dashed line corresponds to curve Δ in (A).

rat femur are smaller by a factor of about two compared to the tibia of the beagle (200 μm) and the femur of man (228 μm) [7]. The hump in the curves for ²⁴¹Am animals, which is located just above the metaphyseal band, is the quantitative evidence of the enlarged trabecular dimensions.

During its shift through the metaphysis the maximum increases and the effect becomes more and more pronounced (Fig.6(A) and (B)). The influence of DTPA can be clearly seen, since for treated animals the rise of the curve between 5 mm and 7.5 mm to the thickness of the compacta indicates that there is no zone of abnormal trabeculation (Fig.6(B)). Comparison with the control reveals, however, that the small calcified tissue remnants normally found in the transition region between metaphysis and diaphysis in the proximity of the compacta are absent. These structures cause the curve for the control to rise gradually to the width of the compacta.

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DISCUSSION

W.S.S. JEE: Do you have any information on lower dose levels and histomorphologic (cellular) data? If not, do you plan to do such work in the future?

E. POLIG: We have no such information as yet. Certainly the ^{241}Am dose applied in this investigation is rather high, and we should reduce the dose to more realistic levels. We intend to do that for ^{239}Pu , and also to extend the period of observation and look at the cell populations.

Miriam FINKEL (Co-chairman): Your study is a beautiful example of the sort of information which can be obtained from a careful study of a few bones. The reason for treatment with DTPA, of course, was to remove ^{241}Am in the hope that this removal would decrease the incidence of osteosarcomas in the event of accidental exposure. Has any work been done on the incidence of bone cancer with and without DTPA treatment? I am aware of the ^{239}Pu work in this area, but wonder if I have missed similar experiments with ^{241}Am .

E. POLIG: I believe some experiments have been carried out by Soviet scientists, showing in the case of ^{239}Pu the influence of DTPA, which indeed reduced the tumour frequency, but not in the case of ^{241}Am .

ESTIMATION OF TUMOUR RISK AT LOW DOSE FROM EXPERIMENTAL RESULTS AFTER INCORPORATION OF SHORT-LIVED BONE-SEEKING ALPHA EMITTERS ^{224}Ra AND ^{227}Th IN MICE *

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Abstract

ESTIMATION OF TUMOUR RISK AT LOW DOSE FROM EXPERIMENTAL RESULTS AFTER INCORPORATION OF SHORT-LIVED BONE-SEEKING ALPHA EMITTERS ^{224}Ra AND ^{227}Th IN MICE.

As a revision of older and recent data of experimental bone-tumour induction in mice after single and repeated incorporation of short-lived ^{224}Ra and ^{227}Th , the following indications for estimation of tumour risk at low dose can be given. (1) Dose rate has considerable influence on tumour risk following internal irradiation, but the relative increase of risk with decreasing dose rate is dose dependent. (2) At a tumour risk of about 10% the range of incidence in repeated experiments is high. It therefore seems difficult to find the exact dose-effect relationships in low-risk experiments. (3) On the other hand, the lower incidence at lower dose level seems to be, rather, a risk of the second half of lifespan. In addition, the high range of the rate of spontaneously occurring neoplasms at greater age does not permit exact statements about the whole lifespan. (4) However, one can tentatively assume that it should be possible to find dose limits for the tumour risk during the first half of the lifespan. (5) Besides radiation dose, other cofactors may interfere at the low-dose and low-risk level. There is no significant decrease in osteosarcoma risk if the incorporation period starts after stunting of growth. The greater risk in female mice than in males indicates hormones as an example of cofactors for the pathogenesis of osteosarcoma.

INTRODUCTION

In estimating the tumour risk after incorporation of short-lived bone-seeking alpha-emitting radionuclides at the low dose level, three main problems arose.

The first is the variation of the incidence of spontaneous and induced neoplasms. The second is the influence of time distribution of dose. The third is the time function of the tumour risk during the life span of an organism. We will discuss these problems by revision of older experimental data [1 - 3] and by comparison with more recent results of long-term experiments in mice after incorporation of ^{224}Ra and ^{227}Th . For ^{224}Ra these problems have been already discussed by Spiess and Mays [4, 5].

MATERIALS AND METHODS

Experimental details of the long-term experiments have already been published by Müller et al. [3]. Most of the experimental data refer to

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female NMRI mice, 3-4 weeks old at the beginning of the experiment. Only a few experiments were also done with male NMRI mice of the same age, with adult female NMRI mice (5-6 months old) and with (C3H x 101)F₁ males and females 3-4 weeks old.

Both strains have been kept in Neuherberg for about ten years. ²²⁴Ra (half-life 3.64 days) was used as chloride and ²²⁷Th (half-life 18.7 days) as citrate. The radionuclides were injected intraperitoneally as isotonic solutions.

Data of the following experimental groups are presented:

Single injection (mean dose to the skeleton in brackets)

²²⁴Ra series:

1.0 μ Ci/kg (30 rad); 2.5 μ Ci/kg (75 rad); 5.0 μ Ci/kg (150 rad);
10.0 μ Ci/kg (300 rad); 12.0 μ Ci/kg (360 rad); 36.0 μ Ci/kg (1080 rad).

²²⁷Th series:

0.1 μ Ci/kg (20 rad); 0.5 μ Ci/kg (100 rad); 1.0 μ Ci/kg (200 rad);
5.0 μ Ci/kg (1000 rad); 10.0 μ Ci/kg (2000 rad).

TABLE I. SPONTANEOUS OCCURRENCE OF NEOPLASMS OF THE LYMPHORETICULAR TISSUE IN FEMALE NMRI MICE

Results from 6 control groups (49 - 199 mice per group) since 1968

Time ^a Period (months)	Mean value					Minimum ^b					Maximum ^b				
	n	N	%	Pl	Pr	n	N	%	Pl	Pr	n	N	%	Pl	Pr
1 - 3	0	643	0.0	0.0	0.6	0	199	0.0	0.0	1.8	0	49	0.0	0.0	7.3
4 - 6	0	643	0.0	0.0	0.6	0	199	0.0	0.0	1.8	0	49	0.0	0.0	7.3
7 - 9	1	637	0.2	0.0	0.9	0	195	0.0	0.0	1.9	1	198	0.5	0.0	2.8
10 - 12	4	620	0.7	0.2	1.7	0	50	0.0	0.0	7.1	1	93	1.1	0.0	5.9
13 - 15	12	592	2.0	1.1	3.5	0	89	0.0	0.0	4.1	2	48	4.2	0.5	14.3
16 - 18	33	545	6.1	4.2	8.5	6	154	3.9	1.4	8.4	6	47	12.8	4.8	25.7
19 - 21	68	460	14.8	11.8	18.6	6	68	8.8	3.3	18.2	10	38	26.3	13.4	43.1
22 - 24	73	329	22.2	18.2	27.5	16	105	15.2	9.0	23.6	11	21	52.4	29.8	74.3
25 - 27	59	189	31.2	24.7	38.4	7	35	20.0	8.4	36.9	5	13	38.5	13.9	68.4
28 - 30	21	77	27.3	17.7	38.6	4	17	23.5	6.8	49.9	5	17	29.4	10.3	56.0
31 - 33	3	23	13.0	2.8	33.6	-	--	--	--	--	2	13	15.4	1.9	45.5
34 - 36	[2]	[3]	--	--	--	-	--	--	--	--	-	-	--	-	--
1 - 36	276	643	42.9	39.3	47.3	33	97	34.0	24.7	44.3	32	50	64.0	49.2	77.1

n = no. of mice with neoplasm.

N = no. of mice living at beginning of specific time interval.

Pl and Pr = 95% confidence limits estimated for a binomial distribution.

^a Observation period started at 1 month of age.

^b If n = 0 the group with largest N has been taken for minimum and the group with smallest N for maximum. Single values were not used if N reached values lower than 10.

Repeated injections (maximum dose rate to the skeleton in brackets)²²⁴

Ra series:

2 x 0.5 μ Ci/kg per week (8 rad/d) for 4, 12, 36 weeks,2 x 1.5 μ Ci/kg per week (24 rad/d) for 4, 12, 24 weeks.²²⁷

Th series:

2 x 0.28 μ Ci/kg per 4 weeks (5 rad/d reached at about 10 weeks after start of the injection period) for 36 weeks.

Each dose group included at least about 50 animals. The total number of control animals is about 850 females and 250 males for the NMRI and 50 males and 50 females for the (C3H x 101)F₁. The mice were examined 6 out of 7 days per week. Autopsy and X-ray examination were performed on all dead or moribund killed animals.

For standardization of results only these osteosarcomas were used for the comparison of incidences in different groups which could be detected on the X-ray picture. Except the series with repeated injections of ²²⁷Th, all tumours were confirmed histologically.

TABLE II. SPONTANEOUS OCCURRENCE OF OSTEOSARCOMAS^a IN FEMALE NMRI MICE

Results from 11 control groups (25 - 199 mice per group) since 1966

Time Period ^b (months)	Mean value					Minimum ^c			Maximum ^c		
	n	N	%	Pl	Pr	n	N	%	n	N	%
1 - 3	0	892	0	0.0	0.4	0	199	0	0	25	0.0
4 - 6	0	891	0	0.0	0.4	0	199	0	0	25	0.0
7 - 9	0	884	0	0.0	0.4	0	198	0	0	24	0.0
10 - 12	0	883	0	0.0	0.4	0	190	0	0	23	0.0
13 - 15	2	818	0.2	0.0	0.9	0	177	0	1	89	1.1
16 - 18	1	761	0.1	0.0	0.7	0	154	0	1	177	0.6
19 - 21	2	648	0.3	0.0	1.1	0	147	0	1	76	1.3
22 - 24	1	467	0.2	0.0	1.2	0	106	0	1	15	6.7
25 - 27	0	278	0.0	0.0	1.3	0	71	0	0	10	0.0
28 - 30	0	130	0.0	0.0	2.8	0	35	0	0	11	0.0
31 - 33	0	43	0.0	0.0	8.2	0	13	0	-	--	--
34 - 36	0	12	0.0	0.0	26.5	-	--	-	-	--	--
1 - 36	6	884	0.7	0.2	1.5	0	50	0	1	30	3.3

n = no. of mice with osteosarcoma.

N = no. of mice living at beginning of specific time interval.

Pl and Pr = 95% confidence limits estimated for a binomial distribution.

^a

Osteosarcomas of the jaws excluded.

^b

Observation period started at one month of age.

^c

If n = 0 the group with largest N has been taken for minimum and the group with smallest N for maximum. Single groups were not used if N reached values lower than 10.

During the early experiments of the whole series the vascular type of osteosarcoma, with a very low amount of osteoid, was excluded since it was found with very low incidence in experimental groups and in controls. Since the occurrence of this tumour with increased incidence was observed more recently in some experimental groups, the whole series was revised and this type of osteosarcoma also included in the evaluation. With regard to the comparison in human beings, tumours of the jaws in mice as a special effect in rodents were excluded from the evaluation.

To give a rough impression of the validity of the incidence values in groups with different numbers of animals at risk, we used the 95% confidence limits calculated for a binomial distribution [6]. The age-specific incidence, i.e. the tumour rate per three-month intervals, was only calculated if there were at least 10 surviving animals.

RESULTS

1. Variation of frequency of spontaneous occurring neoplasms

Table I shows for female NMRI mice that at older age the age-specific incidence of neoplasms of the lymphoreticular tissue (the most frequent spontaneous neoplasm in our NMRI strain) has a large range of incidence in control groups of different experimental series. But for the first year of life the incidence is nearly zero for all series. This may indicate the uncertainty of statements with regard to small increments of tumour risk with dose, especially if the observation period is extended to the whole life span.

Table II shows the spontaneous occurrence of osteosarcomas. A mean value of less than 1% osteosarcomas was found in nearly 900 female NMRI mice. Occurrence of osteosarcomas during the first year of life has a very low probability and has never been observed until now. But the highest incidence observed in a single group was 1/30.

2. ²²⁴Ra single injection, female NMRI mice

From the data in Table III one can see that in the dose range between 30 rad and about 1100 rad the incidence of osteosarcomas ranges from 7% to over 20%, but without clear mathematical function of dose dependence. The range of incidence in experiments of different time periods is relatively large. At this relatively low tumour risk the variation is also high for age-specific tumour rates, which are not presented in detail here. On the other hand, Fig. 1 demonstrates the increase of the per cent incidence per 100 rad with decreasing dose for the dose ranges lower than 300 rad, where it reaches 10% to 20% per 100 rad. The time distribution of tumour risk is not significantly different at various dose levels of this low dose range.

3. ²²⁷Th single injection, female mice

Below 1000 rad mean skeletal dose there is a steep increase of the osteosarcoma incidence with dose, which reaches nearly 50% and more for 1000 rad (Table IV). In groups with such a high incidence the range of incidence in repeated experiments is relatively small.

TABLE III. ^{224}Ra SINGLE INJECTION, FEMALE NMRI MICE
OSTEOSARCOMA^a INCIDENCE IN DIFFERENT DOSE RANGES AND
REPEATED EXPERIMENTS AT THE SAME DOSE

Exp.	Start ^b	$\mu\text{Ci/kg}$	MSD (rad)	n	N	%	Pl	Pr
RG	XI/68	1.0	30	14	199	7.0	3.9	11.6
RG	XI/68	2.5	75	23	199	11.6	7.5	16.9
RC	IV-VI/66	5.0	150	11	50	22.0	11.5	36.0
RCB	X-XII/66	5.0	150	10	48	20.8	10.5	35.0
RF-66	X-XII/66	5.0	150	2	19	10.5	1.3	33.1
RF-67	I-IV/67	5.0	150	2	20	10.0	1.2	31.7
RC	IV-VI/66	10.0	300	6	49	12.3	4.6	24.8
RCB	X-XII/66	10.0	300	4	49	8.2	2.3	19.6
RF-66	X-XII/66	10.0	300	3	20	15.0	3.2	37.9
RF-67	I-IV/67	10.0	300	5	24	20.8	7.1	42.2
RKB	VIII/70	12.0	360	11	49	22.5	11.8	36.6
RKB	VIII/70	36.0	1080	4	50	8.0	2.2	19.2
RKE	II/73	36.0	1080	7	50	14.0	5.8	26.7

MSD = mean skeletal dose.

n = no. of mice with osteosarcoma.

N = no. of mice investigated.

Pl and Pr = 95% confidence limits estimated for a binomial distribution.

^a Osteosarcomas of the jaws excluded.

^b Time of start of the observation period at 1 month of age (Roman numbers = months).

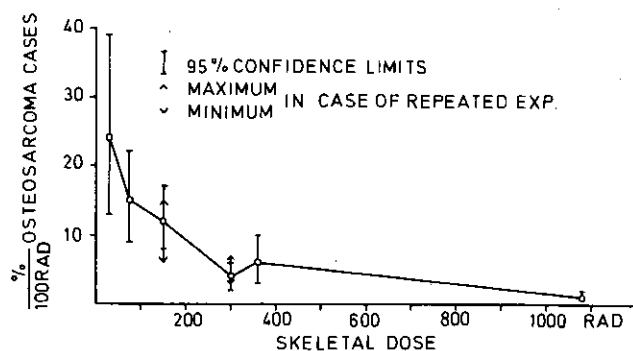


FIG.1. ^{224}Ra single injection, female NMRI mice. Per cent incidence of osteosarcoma cases per 100 rad at different dose levels.

TABLE IV. ^{227}Th SINGLE INJECTION, FEMALE MICE
OSTEOSARCOMA^a INCIDENCE IN DIFFERENT DOSE RANGES AND
REPEATED EXPERIMENTS AT THE SAME DOSE

Exp	Strain	Start ^b	$\mu\text{Ci/kg}$	MSD (rad)	n	N	%	Pl	Pr
THB	NMRI	IV/70	0.1	20	3	99	3.0	0.6	8.6
THB	NMRI	IV/70	0.5	100	11	100	11.0	5.6	18.8
THB	NMRI	IV/70	1.0	200	22	100	22.0	14.3	31.4
THB	NMRI	IV/70	5.0	1000	60	100	60.0	49.7	69.7
THD	NMRI	VII/73	5.0	1000	48	100	48.0	37.9	58.2
THC	(C3H \times 101)F ₁	IV/72	5.0	1000	56	96	58.3	47.8	68.3
THB 10	NMRI	IV/70	10.0	2000	48	98	49.0	38.7	59.3
THD 10	NMRI	VII/73	10.0	2000	45	100	45.0	35.0	55.3

MSD = mean skeletal dose.

n = no. of mice with osteosarcoma.

N = no. of mice investigated.

Pl and Pr = 95% confidence limits estimated for a binomial distribution.

^a Osteosarcomas of the jaws excluded.

^b Time of start of the observation period at 1 month of age (Roman numbers = months).

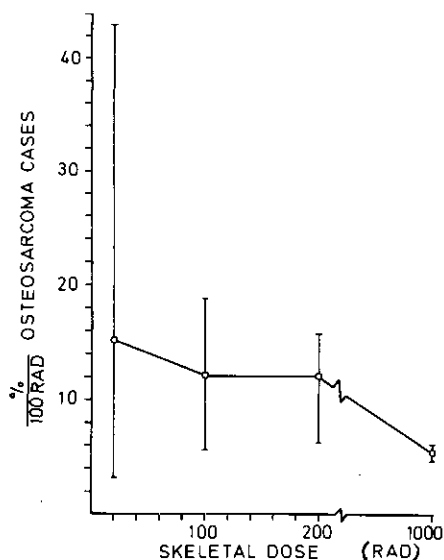


FIG.2. ^{227}Th single injection, female NMRI mice. Per cent incidence of osteosarcoma cases per 100 rad at different dose levels.

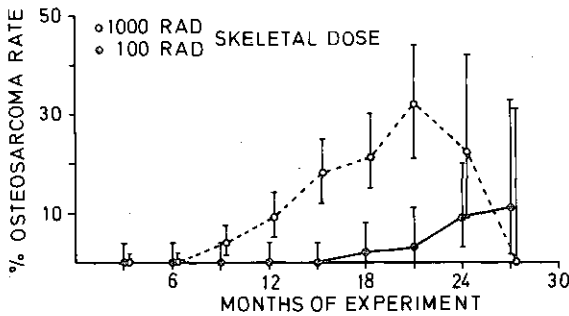


FIG. 3. ^{227}Th single injection, female NMRI mice. Time function of osteosarcoma rate at low and high dose to the skeleton.

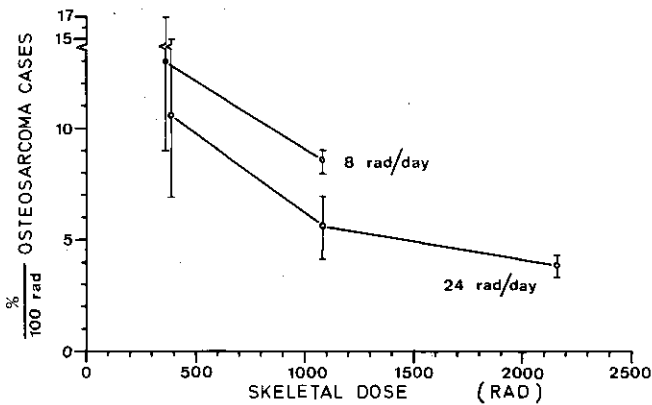


FIG. 4. ^{224}Ra repeated injections, female NMRI mice. Incidence of osteosarcoma cases (%/100 rad) as a function of dose at two different levels of maximum skeletal dose rate:

- (a) 8 rad/d, i.e. $2 \times 0.5 \mu\text{Ci/kg}$ per week;
 (b) 24 rad/d, i.e. $2 \times 1.5 \mu\text{Ci/kg}$ per week.

Even for the $(\text{C3H} \times 101)\text{F}_1$ females the result is very similar. This strain has, in contrast to the NMRI, a very low spontaneous risk of leukemia. Spontaneous osteosarcoma incidence was 1/50 in females.

For the NMRI females the per cent incidence of osteosarcomas per 100 rad increases with decreasing dose below 1000 rad to values of more than 10%/100 rad (Fig. 2). But at the lower dose level the maximal age-specific tumour rate occurs during the later life period as compared to the higher dose level (Fig. 3).

4. ^{224}Ra repeated injections, female NMRI mice

Figure 4 summarizes the results of several experiments with regard to the dose dependence of the per cent incidence per 100 rad. The risk increases with decreasing dose below 1000 rad to values of more than 10%/100 rad. In addition the risk is higher for the lower maximum dose rate 8 rad/d. But for the groups with 360 rad total dose this difference

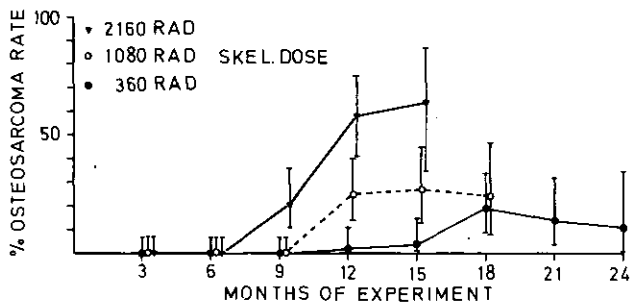


FIG.5. $2 \times 1.5 \mu\text{Ci } ^{224}\text{Ra/kg}$ per week (corresponding to maximum skeletal dose rate 24 rad/d), female NMRI mice. Time function of osteosarcoma rate at total mean skeletal doses 360, 1080, 2160 rad.

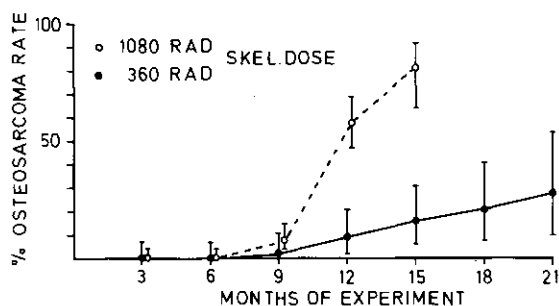


FIG.6. $2 \times 0.5 \mu\text{Ci } ^{224}\text{Ra/kg}$ per week (corresponding to maximum skeletal dose rate 8 rad/d), female NMRI mice. Time function of osteosarcoma rate at total mean skeletal doses 360 and 1080 rad.

is not significant. Consequently the increment of risk per dose unit with decreasing total dose is not higher at the lower dose rate.

Also for the experiments with dose protraction, the age-specific tumour rate shows very clearly the steeper increase of the tumour risk at earlier periods in the higher dose as compared to 360 rad, which is demonstrated for both dose rates used in Figures 5 and 6.

Corresponding values can be calculated for the incidence per 100 rad. For the series with 8 rad/d during 10 to 12 months after start of treatment period, the values were more than 5%/100 rad for the 1080-rad dose group and below 3%/100 rad for the 360-rad dose group.

5. Influence of age

A total amount of $5 \mu\text{Ci/kg } ^{227}\text{Th/kg}$ was given to 1-month and 5-6-months old NMRI females by individual injections of $0.28 \mu\text{Ci/kg}$ with time interval 2 weeks. The dosimetric data were not significantly different for both groups and resulted in about 1000 rad mean skeletal dose and 5 rad/d maximum dose rate to the skeleton, which was reached within about 10 weeks after start of the injection period.

For both age groups the final osteosarcoma incidence was about 80% (for 1-month age group: $42/50 = 84.0\%$, 95% confidence limits 66-90%; for 5-6-months age group: $76/100 = 76.0\%$, 95% confidence limits

66-84%) within 17 months after start of the injection period, when the last animal was killed moribund. This incidence was also similar to the corresponding experiment in the ^{224}Ra series with 1-month-old NMRI females. The age-specific tumour rate during 10 to 12 months after start is even higher for the greater age.

6. Influence of sex

Three osteosarcomas of low-grade osteoid forming and vascular type were observed in 268 male NMRI control mice, i.e. 1.1% (95% confidence limits 0.2 - 3.3%).

As in earlier experiments with single injection of ^{224}Ra [1-3], the tumour risk was also higher for female NMRI mice than for males in an experiment with dose protraction of 12 $\mu\text{Ci/kg}$ (i.e. 360-rad skeletal dose). Single injection, males: 4/50 = 8.0% (95% confidence limits 2-19%); females: 11/49 = 22.5% (95% confidence limits 12-36.5%). Protraction over 4 weeks (2 x 1.5 $\mu\text{Ci/kg}$ per week), males: 6/50 = 12.0% (95% confidence limits 4.5-24%), females: 19/50 = 38.0% (95% confidence limits 24.5-53%).

For the higher dose range with (C3H x 101)F₁ mice after incorporation of 5 $\mu\text{Ci } ^{227}\text{Th/kg}$ (i.e. 1000 rad-skeletal dose) the relative difference of the final osteosarcoma incidence was not very high (males 41/99 = 41.5%, 95% confidence limits 31.5-52%; females 56/96 = 58.5%, 95% confidence limits 48-68.5%). But there was a significant difference of the tumour rate during 13th to 18th months after incorporation (females higher than 15%; males lower than 10%).

DISCUSSION

The variation of the incidence of spontaneously occurring neoplasms of the lymphoreticular tissue was observed mainly at higher age. Therefore risk estimates limited to the lower age of the animals have more validity. The variation of incidence of spontaneous neoplasms in mice is also known from other laboratories [7-9].

In addition, a high range of the osteosarcoma incidence was also found in low-risk experiments by repeated experiments at the same dose level.

Irrespective of these limitations, one can state that the per cent incidence per 100 rad increases with decreasing dose below 1000 rad and reaches even higher values than calculated from human data by Spiess and Mays [5]. This phenomenon also refers to the problems of the RBE at low dose (for discussion and references see Ref. [10]). Since we have several control groups with osteosarcoma incidence 0%, we, like Spiess and Mays, made no correction for control values.

For certain dose ranges the risk increases with lowering of the dose rate. This also partly explains the higher effectiveness of ^{227}Th than ^{224}Ra [2]. But for the comparison of the maximum dose rates 24 rad/d and 8 rad/d the tumour risk is not significantly different for the lower dose tested, i.e. 360 rad. Therefore it is not surprising that the increment of risk per dose unit with decreasing dose is similar for different levels of dose rate in experiments with repeated injection of ^{224}Ra (i.e. for maximum dose rate 24 and 8 rad/d). If one considers the time distribution of the osteosarcoma risk, it can be seen that the risk at low dose mainly

occurs at greater age. Therefore risk estimates at the low dose level should not only consider the incidence for the whole life span, but also the loss of a considerable part of the life span by the occurrence of neoplasms [10]. A more detailed discussion of the mathematical description of the influence of dose and dose rate on the age-specific tumour risk will be published by O. Hug.

The influence of age at time of incorporation on bone sarcoma induction has already been discussed by Spiess and Mays [4, 5], according to whom, on the basis of approximately equal risk at equal skeletal dose and equal protraction time, the juvenile skeleton is "not unusually sensitive" to osteosarcoma induction. Our results in mice indicate that the beginning of the injection span after the period of growth does not result in a drastically decreased tumour risk. But for our experiments one must take into account that also in the lower age group more than two-thirds of the radiation dose was delivered to the skeleton after stunting of growth. On the other hand, osteosarcoma incidence was considerably high (nearly 80%) for both age groups.

The higher osteosarcoma risk in female mice is not directly comparable to man [4], but it indicates the possible influence of cofactors for the pathogenesis of radiation-induced bone tumours. Nilsson [11] has demonstrated the increased osteosarcoma risk after combined application of estrogen and ^{90}Sr in mice. Especially at the low dose level, cofactors of oncogenesis may be important for radiation-induced tumours.

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DISCUSSION

M. GOLDMAN: As the dose rate declines from 24 to 8 rad/d (i.e. from ~200 to 80 rem/d) and below, is it not reasonable to expect that the effectiveness of tumour induction will at some lower dose rate begin to decline rather than continue to rise, as the two points presented suggest? Such a phenomenon has been seen with other bone-seeking radionuclides in animals and, in the case of ^{226}Ra , in humans.

A. LUZ: This would seem to be indicated tentatively by the fact that the "dose rate" or "dose protraction effect" between 24 and 8 rad/d is only significant at the higher dose level. But to resolve this problem we need to have more knowledge about the pathogenesis of the tumours. Reduced cell-killing does not, for instance, account for the difference between the two levels of dose rate since we cannot find impressive degenerative lesions in these groups, as our first (unpublished) studies in this field showed. Perhaps the studies now under way on the activation of virus-linked oncogenesis at the low dose-rate level will provide part of the answer to the problem.

C.W. MAYS: You and your co-worker Walter Müller are to be heartily congratulated on this experiment, which is undoubtedly the best evidence for the enhanced effectiveness of α irradiation with protraction. This is of particular relevance to the problem of exposure of man to ^{239}Pu , which, because of its long half-life, continues to irradiate the internally contaminated person throughout his life. The protraction effect for α particles is opposite to that for low LET radiation (X-rays, γ rays and β particles), for which the effectiveness decreases as a given dose is protracted at a low dose rate.

LOW-LEVEL DETERMINATION OF SKELETAL ^{228}Ra AND ^{228}Th IN THE PRESENCE OF GROSS AMOUNTS OF ^{226}Ra *

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Abstract

LOW-LEVEL DETERMINATION OF SKELETAL ^{228}Ra AND ^{228}Th IN THE PRESENCE OF GROSS AMOUNTS OF ^{226}Ra .

A method is described for determining ^{228}Th (and hence ^{228}Ra) in small samples of human bone when accompanied by many times as much ^{226}Ra . Such determinations are needed for the radiation dosimetry of skeletally-deposited radium 30-50 years after intake of mixtures in unknown proportions of the two isotopes of radium. The only preparation of the bone sample is ashing and dissolution in nitric acid. The short-lived radioactive gas, ^{220}Rn (54.5 seconds), is de-emanated from the solution with nitrogen, which carries it into a 3.8-litre collection chamber. Positively charged ions of ^{210}Po and ^{212}Pb produced by the decay of the ^{220}Rn during its stay in the chamber are collected on a negatively charged electrode in the form of a copper disk, to which is cemented a zinc sulphide phosphor-coated Mylar disk. De-emanation and ion collection continue for up to 24 hours. The electrode is then removed and presented to a second phosphor disk on the window of a photo-multiplier tube. Alpha particles from the collected radioactivity are thus counted with almost 4π geometry, and an initial efficiency is obtained of about 0.3 count per disintegration of ^{228}Th in the solution. Positively charged ions of ^{218}Po and ^{214}Pb from the decay of some ^{222}Rn in the chamber are also collected. The overall efficiency for this is much lower than for the collection of the daughters of ^{220}Rn but, because of the much higher activity of the ^{226}Ra , the initial counting rate is primarily due to its daughters. To make maximum use of all the counting data, they are analysed by a computer method of least squares. In routine use, de-emanation for 1400 minutes, followed by counting for 1400 minutes, permits the measurement of a few tens of fCi of ^{228}Th . The need for correct weighting of very low counting rates is demonstrated.

Introduction

The admixture of ^{228}Ra (MsTh1 , 5.75 years) with ^{226}Ra has long been recognized as a complicating factor in the radiation dosimetry of radium poisoning [1]. Forty years after entry into the blood of 1 μCi of each of these isotopes of radium, approximately equal average radiation doses to the skeleton will have accrued (10.7 rad and 8.5 rad from ^{228}Ra and ^{226}Ra , respectively), but the average concentrations of the two nuclides in bone will be 0.007 pCi/g and 1 pCi/g respectively. Thus special efforts are necessary to make reliable measurements of the very small amounts of activity present in such bones today.

This paper describes a method for the determination of very small amounts of members of the ^{228}Ra decay chain in the presence of much larger amounts of ^{226}Ra . While it was developed, and is used, primarily for bone samples, it is equally applicable to other biological samples.

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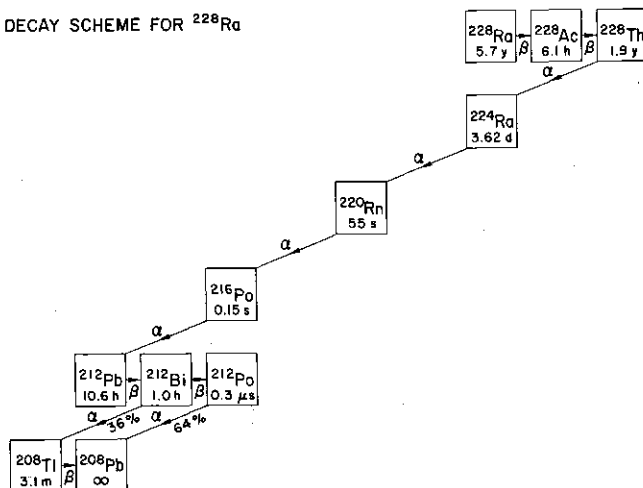
DECAY SCHEME FOR ^{228}Ra FIG.1. The 4n radioactive decay chain, starting with 5.75-year ^{228}Ra (MsTh1).

Figure 1 shows the decay chain of the 4n radioactive series, starting with ^{228}Ra instead of ^{232}Th , which is of no concern here. Radiochemical methods are available in our laboratory for the determination of the longer-lived members of the chain (^{228}Ra , ^{228}Th , ^{224}Ra), but for very low-level work the possibility of contamination by traces in the reagents of the radioisotopes being sought is very real. Such methods may also be time-consuming. The method we have adopted consists of de-emanation of the 55-second radioactive gas ^{220}Rn (thoron) from a solution containing the sample, and electrostatic collection of the charged ions of ^{216}Po and/or ^{212}Pb . The only preparation of the sample is ashing and dissolution in nitric acid. Such a method has been used with various modifications for 40 years [2]; in our version we have exploited recent instrumental and technical developments and the capability of modern computers, to the point where we can process four samples a day routinely and measure levels of a few tens of fCi of ^{228}Th . Progress in our development of the method has been reported [3, 4].

In order to calculate the amount of ^{228}Ra in a sample from the amount of ^{228}Th found by this method, the latter must be divided by the activity ratio $^{228}\text{Th}/^{228}\text{Ra}$. For samples in transient equilibrium (e. g. bones from persons who died more than about five years before the analysis) this ratio is determined from the half-lives for radioactive decay, and is given by

$$\frac{5.75}{5.75 - 1.91} = 1.497$$

For bone from a recently deceased person, allowance must be made for the fact that this ratio may be different because of the different rates of loss of the two elements from bone. This means that the calculation of

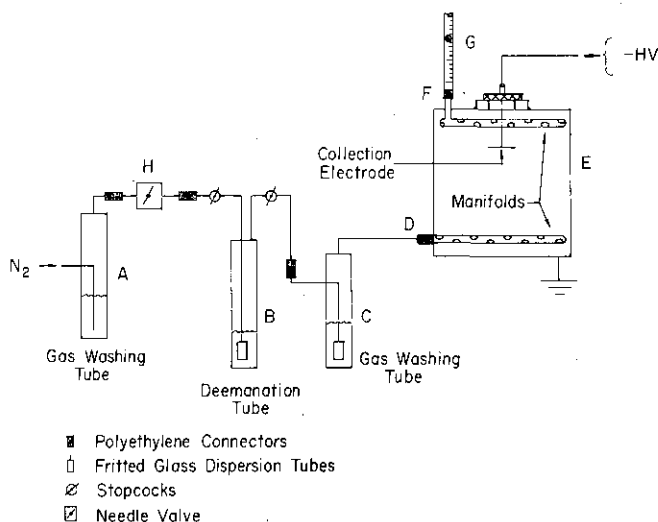


FIG.2. Schematic diagram of apparatus used for the de-emanation of 55-second ^{220}Rn (thoron) and collection of its solid daughters.

the ^{228}Ra content can only be made from at least two estimates of the ^{228}Th content separated in time by about a year. This is the principal disadvantage of the method compared with the radiochemical one which, however, has a higher limit of detection as it is based on β -particle counting of the ^{228}Ac daughter.

De-emanation and Ion Collection

A schematic diagram of the apparatus used for the de-emanation of the thoron and the collection of the ions is shown in Figure 2. The thoron is de-emanated from the solution by passing nitrogen through it. The volume of the acid solution of the sample is adjusted to 100 ml, and it is placed in a 250-ml gas washing bottle (B) with a fritted cylinder of coarse porosity. The nitrogen is first humidified by its passage through distilled water in gas washing bottle A and the flow rate is controlled by a needle valve H at the exit from this bottle. The de-emanation bottle B has a loose-fitting plug of glass wool at the outlet to trap spray from the sample solution and the gas then passes through a second washing bottle C to remove acid vapors, via a manifold D into a 3.8-liter collection chamber E, and is exhausted to atmosphere through the outlet F of another manifold and a calibrated flowmeter G.

For a gas flow-rate in the range of 1-2 liters/min, the average residence time in the 3.8-liter collection chamber is 1.9-3.8 minutes, i.e. two or more half-lives of thoron. Thus at least 75% of the thoron entering the collection chamber will decay there, and the ions of ^{216}Po (half-life 0.15 s) and ^{212}Pb (half-life 10.6 h) will be available for collection on the negatively charged electrode. This electrode, shown

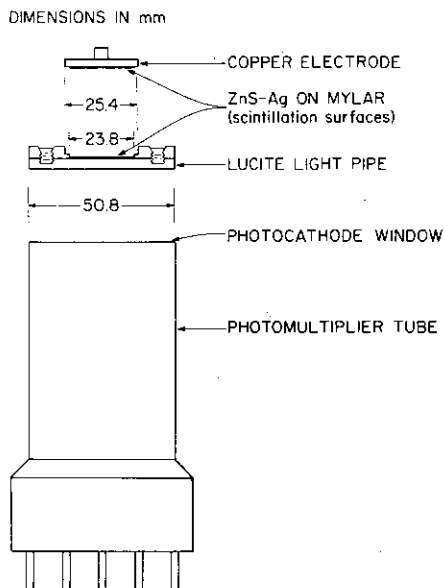


FIG. 3. Scintillation counter used for measurement in 4π geometry of the α activity due to the daughters of ^{212}Pb (ThB) collected on the lower surface of the copper electrode.

in a little more detail in Figure 3, consists of a 25.4 mm diameter copper disk, which is screwed to a 25 mm long brass rod attached to a standard high voltage connector. During the de-emanation and collection the electrode is maintained at a potential of 3.6 kV negative with respect to the grounded metal collection chamber. A 24 mm diameter disk of Mylar, 0.013 mm thick, coated with 4.35 mg/cm^2 of zinc sulfide phosphor, is attached to the face of the copper disk with a small quantity of transparent silicone grease. The positively charged ions deposit on this phosphor disk.

Samples for analysis commonly contain several hundred times as much ^{226}Ra as ^{228}Th (or ^{228}Ra), and this is a constant source of ^{222}Rn (half-life 3.823 d). The solid daughters of this radioactive gas, ^{218}Po , ^{214}Pb , and ^{214}Bi , will also be collected if they are produced in the collection chamber. For a mean residence time in the chamber of a few minutes, very little ^{222}Rn decays, but the production rate is usually so much greater than that of thoron (^{220}Rn) that most of the activity on the phosphor disk at the end of the collection period is due to the daughters of ^{222}Rn . These decay, with half-lives of 3.1 min, 26.8 min and 19.7 min respectively, to insignificance in about 400 minutes. The remaining activity is due to 10.6-h ^{212}Pb , which can be assayed via its alpha-emitting daughters.

Selection of Operating Conditions

A range of values for the gas flow-rate of 1-2 liters/min has already been mentioned. If the rate is too low, much of the thoron will

decay in the "dead volume" before it gets to the collection chamber, while if it is too high the fraction of thoron which decays in the collection chamber will be reduced, giving a lower efficiency. Evans [2] derived an equation which predicts the variation with gas flow-rate, of the fraction f_λ , of thoron atoms which leave the solution and decay in the collection chamber. However, his expression is based on the assumption that there is no mixing of the incoming gas with that already in the chamber. A. T. Keane has derived a different equation (see Appendix I) for f_λ with the assumption of complete mixing of the incoming gas with the gas already in the chamber. It is found that for a collection chamber volume of 3.78 l and a "dead volume" of 0.376 l, between the surface of the sample solution and the manifold in the collection chamber, the optimum flow-rate is 1.2 l/min from Evans' equation or 0.91 l/min from Keane's equation. Of those thoron atoms which leave the solution, the fraction which decays in the chamber is 0.716 (Evans) or 0.578 (Keane) at these flow-rates. Both these quantities vary only slowly with flow-rate within ± 0.2 l/min of the optimum value. Thus a very stable flow-rate is not necessary.

There is also an optimum value for the voltage on the collection electrode, but this is less amenable to calculation and a value of 3.6 kV was determined experimentally. Below 3.6 kV, the collection efficiency drops at an approximate rate of 1% per 100-volt decrease. There is a smaller decrease in the collection efficiency between 4.0 kV and 5.0 kV (the highest voltage used) of about 0.44% per 100-volt increase. Thus variations in the actual voltage of the supply unit of ± 10 volts have a negligible effect on the collection efficiency at 3.6 kV.

The reason for the decrease in collection efficiency as the voltage exceeds 4 kV is not clear. It may be due to an increase in the number of ions collected on the back (top) surface of the collection disk, and on the supporting rod, at the expense of those collected on the phosphor-covered (bottom) surface of the disk. At 3.6 kV the activity on the top surface does not exceed 10% of that on the bottom surface.

Measurement of the Alpha-activity

At the end of the de-emanation, the high voltage is switched off, and the nitrogen flow is stopped. The collection electrode assembly is removed from the can and the copper disk is unscrewed and transferred to a transparent plastic (Lucite, Plexiglas) light guide, shown in Fig. 3.

A second 24-mm diameter phosphor-Mylar disk is secured with transparent silicone grease in the central countersunk area and the collection electrode is placed in position above it and separated from it by about 0.8 mm. The plastic light pipe is placed on the window of a 5-cm diameter photomultiplier and a light-tight cover is placed over the whole assembly. In this way, the counting geometry is nearly 4π , a feature which offers considerable advantages. Four of these counters and four de-emanation assemblies have been built, for routine use. A single high-voltage source supplies the photomultipliers via switches which must be turned off before the light-tight cover is removed.

Pulses from each photomultiplier are amplified and passed to a discriminator; those pulses which exceed the discrimination setting are counted with a scaler. At the end of a preset time, the scaler readings are automatically recorded on a teletypewriter, with punched paper tape output; the scalers are reset and a new counting interval starts. The time between the end of one interval and the start of the next is quite negligible ($0.2 \mu\text{s}$) compared with the length of the counting time (10–100 min). All four counters are controlled by a single timing unit and the results are printed and punched on tape sequentially, although the counting periods are simultaneous.

The background counting-rates of these counters are in the range 0.015 count/min to 0.03 count/min; the contribution of electronic "noise" is less than 5% (1 count/d).

Calibration and Efficiency

Several sources are used for calibration of the equipment. A solution containing ^{228}Th was prepared by separation from ^{228}Ra in 1965. Residual radium was removed by ion exchange in 1971. The solution was standardized by gamma-ray counting both a "point" source prepared from an aliquot, and a National Bureau of Standards standard source in the same geometry. Another aliquot was diluted to 100 ml and is used as a standard source for the de-emanation method. The activity of this source was 1050 pCi in April 1975, but it suffers from the disadvantage of decreasing with the 1.91-yr half-life of ^{228}Th .

A source which does not have this disadvantage was prepared in 1947 at the Radioactivity Center of the Massachusetts Institute of Technology from thorium nitrate which had been purified before 1907, so that all the daughters are now in equilibrium. This was standardized by chemical analysis for thorium, after a known weight of the thorium nitrate had been ignited to ThO_2 . A solution considered to contain 20.4 mg of thorium (2230 pCi) is used as a second standard source. The efficiency of the de-emanation method as determined with this standard is about 6% higher than the efficiency as determined with the first source, but we do not regard this discrepancy as serious.

A third source is used for experimental studies in which the operating conditions are varied, and where short de-emanations and high counting rates are required. It contains 599 mg of thorium nitrate which was purified in 1958, and the activity of the ^{228}Th in April 1975 was 51 nCi (about 78% of the equilibrium value). This figure has to be revised upwards with the passage of time, as the activities of the ^{228}Ra and ^{228}Th grow towards equilibrium, but this is not a big disadvantage.

None of these standards contains detectable ^{226}Ra .

During the period of de-emanation, the activity of ^{212}Pb on the collection electrode increases as $1 - \exp(-\lambda\tau)$ where λ is the disintegration constant of ^{212}Pb and τ is the elapsed time. Furthermore, at the end of the collection period, the alpha-particle activity starts to decay with the

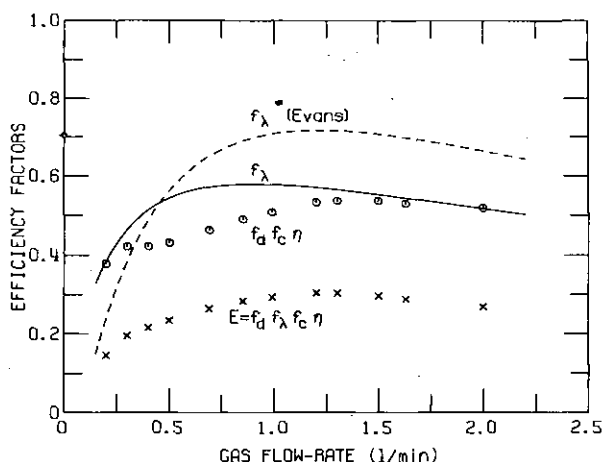


FIG.4. The curves show the fraction of thoron which leaves the solution and decays in the collection chamber, plotted as a function of nitrogen flow-rate, and calculated with the assumptions of no mixing (dashed curve) and complete mixing (continuous curve). The overall efficiency $E(x)$ was determined experimentally and these values were corrected for the decay factor with values from the continuous curve (\odot). (See text and Appendix I.)

half-life of ^{212}Pb , although there may be an initial period during which there is a small increase due to the growth of 1-hour ^{212}Bi . It is therefore convenient to define the overall efficiency E as the ratio of the instantaneous counting-rate at the end of an infinitely long de-emanation, to the disintegration rate of ^{228}Th in solution. Since the relative activity on the collection electrode at any time can be accurately predicted from the equations for growth and decay of the ^{212}Pb and ^{212}Bi (see Appendix II), it is a simple matter to determine the actual activity for a finite period of de-emanation, from the counting-rate observed at any subsequent time.

Values of E were determined at various flow rates and are plotted in Fig. 4 (crosses); a maximum value of 0.304 was observed at a flow rate of 1.2 l/min. The curves in Figure 4 show the calculated variation of the fraction decaying in the collection chamber for Evans' [2] and Keane's equations. E can be expressed as

$$E = f_d f_\lambda f_c \eta$$

where f_d is the fraction of ^{220}Rn removed from the solution by the gas flow (de-emanation efficiency),

f_λ is the fraction of de-emanated ^{220}Rn which decays in the collection chamber,

f_c is the fraction of the ions produced in the chamber which is collected on the phosphor disk (collection efficiency)

and η is the counting efficiency (≈ 1).

By dividing the values of E plotted in Fig. 4 by the calculated values of f_{λ} , we obtain the product $f_d f_c \eta$.

The results of separate experiments suggested that Keane's equation was probably valid at the flow-rate of 1.2 l/min which gave the highest value of E . We therefore divided the experimental values of E by the values of f_{λ} calculated from Keane's equation and the results are also plotted in Fig. 4 (circled points). Since the counting efficiency η is certainly not dependent on gas flow-rate, the variations in the quantity $f_d f_c \eta$ must be due to variations in f_d and/or those in f_c .

We determined f_d at gas flow rates of 0.2, 0.7 and 1.2 l/min, and obtained values of about 0.47, 0.76 and 0.815 respectively. The variations are in respectable agreement with the predictions of Henry's law, with the assumption that the ratio of the concentration of thoron in the effluent gas to the concentration in the solution was constant. By dividing the appropriate values of $f_d f_c \eta$ by these values of f_d we obtain 0.80, 0.61 and 0.65 for $f_c \eta$ at 0.2, 0.7 and 1.2 l/min respectively; the latter two values may not be significantly different. A decrease in this quantity with increasing flow rate may be attributable to ions being swept into the exhaust manifold, which is in a region of weak electrostatic field, before they can be collected. The important fact is that f_d does not vary markedly with flow rate in the region of 1.2 l/min.

Data Processing

In routine use, for the analysis of samples expected to contain low levels (< 10 pCi) of ^{228}Th , de-emanation is continued for 12-24 hours. Counting of the activity on the four phosphor disks starts less than 10 minutes after the end of the collection, and preset counting times of 10 minutes are used initially. After 350-450 minutes, the counting times are increased to 100 minutes. Twenty-four hours after the start of counting, the teletypewriter chart is examined; if the counts accumulated indicate that previously determined values of the counter backgrounds can be used without increasing significantly the statistical errors on the results, counting is terminated. For those cases where the counting rates are so low that a background determination is desirable, counting is allowed to continue for two or three more days. By recording the counts at 10-minute intervals initially, the decay of the daughters of ^{222}Rn can be followed in detail.

The data are analysed by a computer method of least squares [5]. An equation (see Appendix II) which describes the decay of the daughters of ^{222}Rn , the growth of ^{212}Bi and the decay of ^{214}Pb , is fitted to the data and the results are expressed as activity (in counts/min) of ^{214}Pb at the end of collection and as activity (in pCi) of ^{224}Ra in solution. When the background is not determined (24-hour counting time) it is included as a constant in the function. For the longer counting times, the background rate is included as a variable. When the efficiency of the system is to be determined from measurements of a standard, no contribution from the daughters of ^{222}Rn is included in the equation and the background is held constant.

For samples containing the highest ratio of $^{226}\text{Ra}/^{228}\text{Ra}$ activities, the initial counting rate due to the daughters of ^{222}Rn may be two orders of magnitude higher than the final background counting rate. Furthermore, when the average counting rate is only about 0.02 count/min, there is a significant probability of observing no counts in a 100-minute interval. This probability can be calculated according to the Poisson distribution and is 22% for a rate of 0.015 min^{-1} ; it is still 5% for a rate of 0.03 min^{-1} . Thus we expect a number of the counting intervals to contain zero counts and we must consider how the data should be weighted in the least squares fitting procedure. It is customary to use as a weight the inverse of the variance, and the variance here is the number of counts in each counting interval divided by the square of the counting time. Obviously the use of such a weighting procedure would have disastrous consequences in the execution of a computer program which detects zero as the number of counts in an interval. To obviate this problem we increased the number of counts in each interval by one before calculating the weight as $T^2/(N + 1)$ where T is the counting time and N the number of counts.

This procedure appeared to give satisfactory results. However, for those samples which contained very little ^{228}Th and for which counting was continued for several days, it was possible to calculate by hand the background from the counts observed in the last 1000 or 2000 minutes, after ^{212}Pb had ceased to contribute. This value was always significantly higher than the value computed by the program; this is because too much weight was given to the lowest counts, whereas equal weight should be given to all counting rates at times when the average rate is not changing. Dr. A. Jaffey pointed out that the best estimate of the weight is the inverse of the variance of the expected number of counts. We therefore modified the computer program so that the results for each sample were first computed with the weights set to $T_i^2/(N_i + 1)$, and are then recomputed with the weights set to T_i^2/N_i where N_i is the expected number of counts (not an integer) in the i -th counting interval from the first fitted curve. A second repeat of the least squares analysis is then made with the weights set to T_i^2/N_i'' where N_i'' is the expected number of counts from the second fitted curve. The results from this third regression analysis differ hardly at all from those of the second but the variance ratio and the χ^2 probability always indicate much better fits. As an example of the effect and importance of this re-weighting procedure, the results in Fig. 5 and Table I may be considered. The figure shows the counting rates observed after a 16-hour de-emanation of a sample of bone; counting started 16 minutes after the end of the ion collection and continued for 7000 minutes. The counting intervals were 15 minutes initially, but were increased several times; the last seven intervals were 360 minutes and this was sufficiently long for there to be no interval with zero counts. Nevertheless the number of counts in each interval ranged from 4 to 13, with a corresponding range of weights between 25920 and 9993. The fit from the first regression analysis is shown as a dashed curve, and the results are in the first line of Table I. It can be seen from Fig. 5 that there are fewer points below the dashed curve (19) than above it (39) and in line 1 of Table I we note the rather low χ^2 probability. The results of the repeated regression analyses in lines 2 and 3 of Table I are very similar to one another (although they differ markedly from those in line 1), but the statistical

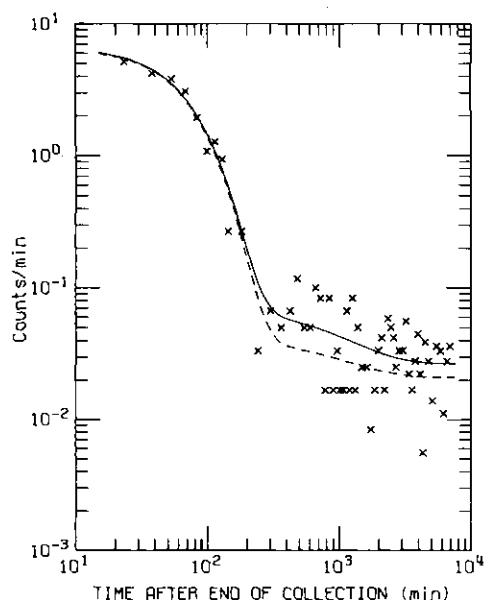


FIG. 5. Observed counting rates after de-emanation for 960 minutes of a solution containing 47 nCi ^{226}Ra and 116 fCi ^{228}Th and the computer-derived results of least-squares fitting with incorrect (dashed curve) and correct (continuous curve) weighting of the experimental data.

TABLE I. DEMONSTRATING THE EFFECT OF CHANGING THE WEIGHTING ON THE RESULTS OF REGRESSION ANALYSES (SEE TEXT)

Analysis number	Variance ratio	χ^2 probability (%)	^{228}Th content (fCi)	^{214}Pb activity (c/m)	Background (c/m)
1	1.32	5.5	53 ± 37	6.0 ± 0.4	0.021 ± 0.002
2	1.50	0.8	120 ± 37	6.1 ± 0.4	0.027 ± 0.003
3	1.08	32.9	115 ± 39	6.1 ± 0.4	0.027 ± 0.003

tests indicate that the third analysis gives a much better fit, and this fit is plotted as a continuous curve in Fig. 5. There are about equal numbers of points on either side of this curve, as would be expected for a good fit.

A value for the background can be deduced from the number of counts (57) observed in the last six counting intervals (2160 minutes); the average rate was 0.026 ± 0.003 count/min, in agreement with the value of 0.027 ± 0.003 found in the second and third regression analyses.

It may be noted in passing that the results in Fig. 5 and Table I emphasize that this method is very inefficient for the measurement of

^{226}Ra . By collecting and counting ^{222}Rn , J. Y. Sha found that the bone sample used in this demonstration contained 47 nCi ^{226}Ra , with a statistical standard error of $\pm 1\%$. This is an exceptionally large amount for a small piece of human bone, yet the statistical standard error on the counting rate from the daughters of ^{222}Rn in the present method amounted to $\pm 6\%$. Correspondingly larger errors would be obtained in the analyses of bone samples containing much smaller activities of ^{226}Ra . Nevertheless the example demonstrates the capability of the technique to measure ^{228}Th (and hence ^{228}Ra) in a bone sample which had a $^{226}\text{Ra}/^{228}\text{Ra}$ activity ratio of about 400,000:1.

In conclusion, we have found this technique to be a useful and powerful tool for the measurement of very low levels of ^{228}Th , and hence of ^{228}Ra . It is very simple to use and highly specific, and the optimum operating conditions do not depend critically on the stability of the nitrogen flow rate or the high voltage on the collection electrode.

Acknowledgments

The interface for the read-out system was designed, constructed and tested by J. E. Miranda of the Electronics Division. We thank N. J. Beskid for his help in the early stages of this work, and F. Markun for assistance in the determination of the de-emanation efficiency.

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APPENDIX I - Fractional decay of thoron in the collection chamber and in the lines leading to the chamber, under the assumption of uniform mixing of the flow gas (A. T. Keane)

A. Decay of thoron in the collection chamber

Thoron is produced by the decay of $3.62\text{-d } ^{224}\text{Ra}$, which is the daughter of $1.91\text{-yr } ^{228}\text{Th}$. For a solution containing ^{228}Th which has not been subject to chemical separation for about a month or more, the

activities of these two nuclides are essentially equal, and constant for times which are short compared to 1.91 years. Thus thoron atoms are produced at a constant rate A' , and

$$A' = \lambda_{228\text{Th}} N_{228\text{Th}} = \lambda_{224\text{Ra}} N_{224\text{Ra}}$$

where λ is the appropriate constant of radioactive decay and N is the number of atoms. The rate of delivery of thoron atoms to the collection chamber, A , is proportional to A' . It is assumed that the turbulence of the flow in the chamber is such that each increment of flow gas is evenly dispersed in the chamber so that thoron is maintained at a uniform concentration throughout the chamber volume. The number of atoms of thoron in the chamber will increase after de-emanation starts, to a maximum and virtually constant value. It can be readily shown that after a time longer than about 10 minutes, this maximum value is given by

$$\frac{A}{\lambda_{\text{Tn}} + R/V}$$

where λ_{Tn} is the decay constant of thoron (0.763 min^{-1}), R is the flow rate of the gas (liters/min) and V is the volume (liters) of the collection chamber.

When electrostatic collection of thoron-decay products begins at about 10 minutes after the start of de-emanation, the fraction, f_D , of thoron atoms delivered to the chamber that decays in the chamber is virtually independent of the collection period, and is given by the rate of decay of thoron atoms in the chamber divided by the rate of delivery of thoron atoms to the chamber:

$$f_D = \frac{N_{\text{Tn}} \lambda_{\text{Tn}}}{A} = \frac{A}{\lambda_{\text{Tn}} + R/V} \cdot \frac{\lambda_{\text{Tn}}}{A} = \frac{\lambda_{\text{Tn}}}{\lambda_{\text{Tn}} + R/V}$$

- B. Decay of thoron in the free volume of the vessel containing the sample solution and in the lines leading to the collection chamber

The free volume of the sample vessel and the lines leading to the collection chamber is a small fraction of the volume of the chamber, so the decay of thoron en route to the chamber will be minimal. Thoron atoms in this "dead volume" will reach a maximum and constant number in a time much shorter than 10 minutes. Under the assumption of uniform mixing of the flow gas in the dead volume, the fraction f_S of the thoron atoms de-emanated from the sample that survives decay in the dead volume and is delivered to the collection chamber is the complement of the fractional decay in the dead volume:

$$f_S = 1 - \frac{\lambda_{\text{Tn}}}{\lambda_{\text{Tn}} + R/v} = \frac{R/v}{\lambda_{\text{Tn}} + R/v}$$

where v is the free volume (liters) of the sample vessel and the lines leading to the collection chamber.

The fraction of atoms which leaves the sample and decays in the collection chamber, f_λ , is the product of f_S and f_D ,

$$f_\lambda = f_S f_D = \frac{R/v}{\lambda_{Tn} + R/v} \frac{\lambda_{Tn}}{\lambda_{Tn} + R/v}$$

and this is plotted as a function of R as the continuous curve in Figure 4.

For comparison, the equation derived by Evans [2] can also be expressed as the product of two factors f'_D and f'_S ,

$$\text{where } f'_D = 1 - \exp(-\lambda_{Tn}(v/R))$$

$$\text{and } f'_S = \exp(-\lambda_{Tn}(v/R))$$

The product f_λ is plotted as the dashed curve in Figure 4.

APPENDIX II - Equations for the growth and decay of ^{218}Po , ^{214}Pb , ^{214}Bi (daughters of ^{222}Rn) and of ^{212}Pb and ^{212}Bi .

At the end of de-emanation the amount of the activity collected due to any one of the daughters of either ^{222}Rn or ^{220}Rn depends on the duration of the collection. Although ^{214}Pb is not an alpha-particle emitter its activity must be taken into account because it gives rise to the emission of alpha particles at a later stage in the decay chain. For a collection period lasting τ minutes the relative activities of the three daughters of ^{222}Rn will be:

$$^{218}\text{Po}: 1 - \exp(-0.227\tau)$$

$$^{214}\text{Pb}: 1 + 0.1284 \exp(-0.227\tau) - 1.1284 \exp(-0.0259\tau)$$

$$^{214}\text{Bi}: 1 - 0.0235 \exp(-0.227\tau) - 4.259 \exp(-0.0259\tau) \\ + 3.283 \exp(-0.0352\tau)$$

We designate these as R_A , R_B and R_C respectively. The relative alpha-particle activity at any time t minutes after the end of the de-emanation period can then be written in the form:

$$R_t = R_1 \exp(-0.227t) + R_2 \exp(-0.0259t) + R_3 \exp(-0.0352t)$$

$$\text{where } R_1 = 1.0235R_A$$

$$R_2 = 0.4848R_A + 3.7748R_B$$

$$\text{and } R_3 = R_C - 0.5083R_A - 3.7748R_B$$

For the daughters of ^{220}Rn (thoron) the relative activities during a collection period τ minutes will be:

$$^{212}\text{Pb}: 1 - \exp(-0.00109\tau)$$

$$^{212}\text{Bi}: 1 - 1.105 \exp(-0.00109\tau) + 0.105 \exp(-0.0114\tau)$$

We designate these as T_B and T_C respectively. The relative alpha-particle activity at any time t minutes after the end of the de-emanation period is then

$$T_t = T_1 \exp(-0.00109t) + T_2 \exp(-0.0114t)$$

$$\text{where } T_1 = 1.1053T_B$$

$$\text{and } T_2 = T_C - 1.1053T_B$$

The observed counting rate Y_t can be related to the relative activities of the daughters of ^{222}Rn and ^{220}Rn by the equation

$$Y_t = k_1 R_t + k_2 E T_t + b$$

where k_1 and k_2 are constants, b is the background counting rate and E is the overall efficiency as defined in the body of the paper. This equation is in a convenient form for the determination of k_1 and k_2 (and b) by the method of least squares analysis.

DOSE, DOSE RATE AND AGE PARAMETERS IN ANALYSIS OF RISK FROM BONE-SEEKING RADIONUCLIDES An extrapolation to low levels *

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Abstract

DOSE, DOSE RATE AND AGE PARAMETERS IN ANALYSIS OF RISK FROM BONE-SEEKING RADIONUCLIDES:
AN EXTRAPOLATION TO LOW LEVELS.

Radiation-induced primary bone sarcomas (PBS) and life-span shortening (Δ) were studied as a function of dose, dose rate and time in beagles fed from midgestation to 1.5 years of age diets containing ^{90}Sr Cl_2 in equilibrium with ^{90}Y , or administered ^{226}Ra Cl_2 in eight semimonthly intravenous injections starting at 2, 4 or 14 months of age. PBS and Δ from $^{90}\text{Sr} + ^{90}\text{Y}$ and ^{226}Ra were dependent on interaction of dose rate, dose, age and time after the administration of the radioactivities, and mode of administration with a dynamic non-linear organ system, the skeleton. Incidence of PBS from $^{90}\text{Sr} + ^{90}\text{Y}$ and ^{226}Ra was calculated from life-tables. Probability of greater than 1% PBS incidence was normally distributed with respect to dose and age. Delay period between exposure and tumour incidence was suggested to be proportional to time to 1% incidence of PBS (t_0). For $^{90}\text{Sr} + ^{90}\text{Y}$ dogs, estimated t_0 were 9.4 years, 5.1 years, and 1.5 years for an average daily ingestion of 4, 12 and 36 μCi $^{90}\text{Sr} + ^{90}\text{Y}$ starting from birth, respectively. For ^{226}Ra , estimated t_0 were 3.8, 3.3, 1.7 and 1.3 years for cumulative injections of 3.14, 9.4, 28.1, and 83.6 μCi , respectively. Radiation-induced life-span shortening was estimated from "practical survival age", age at 8% population survival. At a maximum dose rate \dot{D}_{max} of 2.5 rad/d, $^{90}\text{Sr} + ^{90}\text{Y}$ and ^{226}Ra were equally effective in reducing life-span, but for $\dot{D}_{\text{max}} > 2.5$ rad/d, $^{90}\text{Sr} + ^{90}\text{Y}$ was more effective than ^{226}Ra . A dose of 100 rads for radium and 1700 rads for strontium integrated to $T_{\text{max}} = 16.5$ years did not induce a detectable life-span shortening.

INTRODUCTION

One of the biological effects of ^{226}Ra body burden in mammals is injury to bone cells and bone vascular bed [1, 2, 3] precipitating as primary bone sarcomas [1, 4, 5]. Rowland et al. [6] tabulated incidences of sarcomas in man as a function of the initial radium burden and observed that % incidence increased with the quantity of radium acquired. Extrapolation of the observed incidence to lower levels of irradiation is dependent on exposure parameters: dose, dose rate, age at the first administration of the radionuclide, and the exposure period. This report describes the influence of these exposure parameters on induction of primary bone sarcomas and life shortening in beagles with body burdens of $^{90}\text{Sr} + ^{90}\text{Y}$ or ^{226}Ra .

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SUMMARY OF THE $^{90}\text{Sr} + ^{90}\text{Y}$ AND ^{226}Ra EXPOSURE

The details of the experimental design for long-term toxicity and metabolism of $^{90}\text{Sr} + ^{90}\text{Y}$ and ^{226}Ra in beagles at the Radiobiology Laboratory, University of California at Davis, have been published [7-12]. In summary, $^{90}\text{Sr} \text{Cl}_2$, in equilibrium with ^{90}Y , was fed to dogs mixed with their diet from midgestation to young adulthood (1.5 years of age). These beagles are designated by Code "D". For each dose level, a constant ratio of strontium to calcium in the diet was maintained [7]. A group of 44 beagles was given a single injection of $^{90}\text{Sr} + ^{90}\text{Y}$ at 540 days of age. These dogs, designated by codes "S20" and "40", received $3.7 \mu\text{Ci/kg}$ (19 beagles) and $33 \mu\text{Ci/kg}$ (25 beagles), respectively.

Radium-226 chloride was administered in 8 equal semimonthly intravenous injections starting at 435 days of age. These dogs are designated by code "R" (Table I). Six dogs were similarly injected with an average $1.25 \mu\text{Ci } ^{226}\text{Ra/kg}$ of body mass per injection starting at either two or four months of age. These dogs are designated by code "R5X".

Retention, local distribution and dosimetry of $^{90}\text{Sr} + ^{90}\text{Y}$ in the humeri and skeleton of beagles given a single intravenous injection or a daily diet of the radionuclides (in utero to 18 months of age) has been reported [7, 8, 13]. Retention, local distribution, and dosimetry of ^{226}Ra in humeri [13-16] and in the skeleton [13] of beagles given 8 semimonthly intravenous ^{226}Ra injections started at 14 months of age has been also reported.

TABLE I. ^{226}Ra INJECTION SERIES WITH 8 SEMIMONTHLY INTRAVENOUS INJECTIONS STARTING AT 14 MONTHS OF AGE (A_0 is the average total injected radium.)

Treatment Code	Total ^{226}Ra ($\mu\text{Ci/kg}$)	Multiple of R10 level	A_0 (μCi)	Number of Dogs
R00	0.000	0	0	81
R05	0.024	0.33	0.18	46
R10*	0.064	1.0	0.53	38
R20	0.376	6	3.14	41
R30	1.12	18	9.4	39
R40	3.36	54	28.1	41
R50	10.0	162	83.6	38

* This level was computed to represent the beagle equivalent of ten times the Radiation Protection Guide value for man ($0.1 \mu\text{g } ^{226}\text{Ra}$).

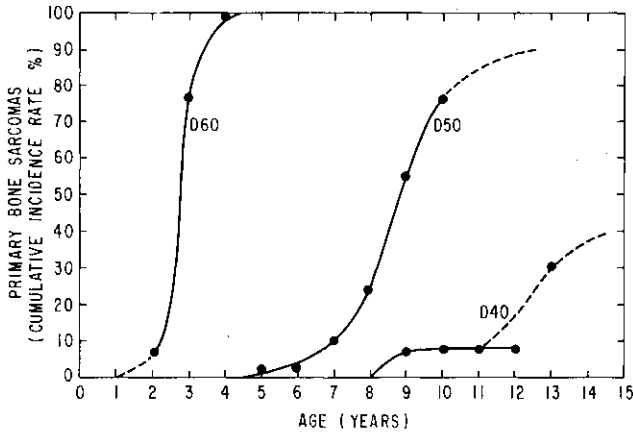


FIG.1a. Cumulative incidence of primary bone sarcomas in beagles ingesting $^{90}\text{Sr} + ^{90}\text{Y}$ from midgestation to 1.5 years of age as a function of age.

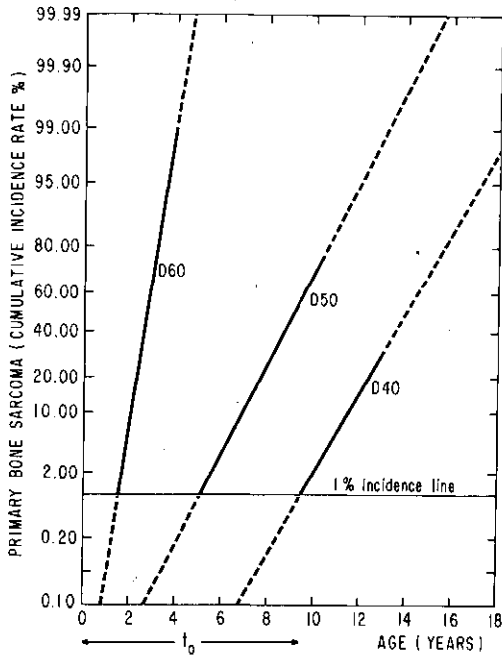


FIG.1b. Cumulative incidence of primary bone sarcomas in $^{90}\text{Sr} + ^{90}\text{Y}$ labeled beagles (see Fig.1a) plotted on a normally distributed probability scale as a function of age. The period between birth to 1% incidence of PBS is designated T_0 .

PRIMARY BONE SARCOMAS IN $^{90}\text{Sr} + ^{90}\text{Y}$ - AND ^{226}Ra - LABELED SKELETONS

Figure 1a shows age-specific cumulative incidence of primary bone sarcomas (PBS), calculated from life-tables [17], in beagles fed $^{90}\text{Sr} + ^{90}\text{Y}$ at D60, D50 and D40 levels. The dotted lines are suggested extrapolations to later ages. Figure 1b gives age-specific cumulative incidence rate of primary bone sarcomas (CPBS), corrected for competitive risk, plotted on a normal probability distribution scale. The normal probability scale was utilized to estimate the distribution of age at death from PBS. CPBS incidence has a normal (straight line) distribution with respect to age at D40, D50 and D60 levels of $^{90}\text{Sr} + ^{90}\text{Y}$ irradiation. The median age of death from PBS (\bar{T} , age at 50% CPBS) and the population standard deviation (σ) were estimated as $\bar{T} = 2.7$ years, $\sigma = 0.55$ years at D60; $\bar{T} = 9.2$ years, $\sigma = 1.7$ years at D50; and $\bar{T} = 14$ years, $\sigma = 2$ years at D40 level. The probability density function, $dP(t)$ is:

$$dP(t) = \left| \frac{1}{\sigma (2\pi)^{\frac{1}{2}}} \right| \exp \left| -\frac{1}{2} (t - \bar{T})^2 / \sigma^2 \right| dt$$

where t is age in years. In these calculations it is assumed that the sample variance (S^2) are estimates of the population variance (σ^2).

The probability density function for PBS as a function of time-integrated dose of $^{90}\text{Sr} + ^{90}\text{Y}$ also appears as a normal distribution except for the D60 dose level (Fig. 2).

Figure 3a shows age-specific cumulative incidence rates of primary bone sarcomas in beagles calculated from life-tables. The curve for R5X is

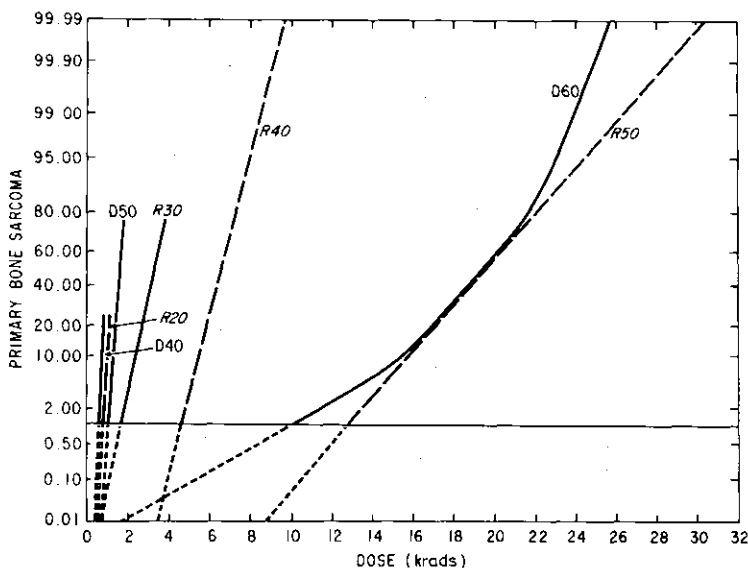


FIG.2. Cumulative incidence of primary bone sarcomas in beagles ingesting $^{90}\text{Sr} + ^{90}\text{Y}$ or injected with ^{226}Ra plotted on a normal probability scale (see Figs 1a, 1b) as a function of time-integrated dose.

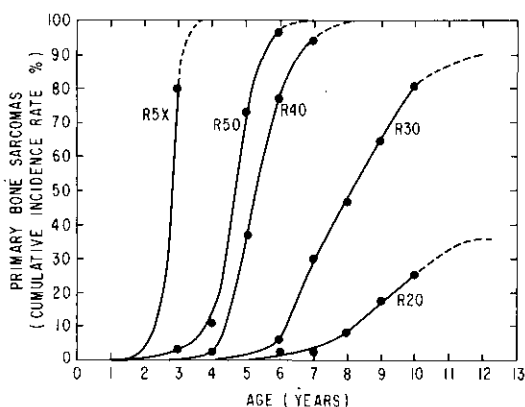


FIG.3a. Cumulative incidence of primary bone sarcomas in beagles injected with ^{226}Ra as a function of age.

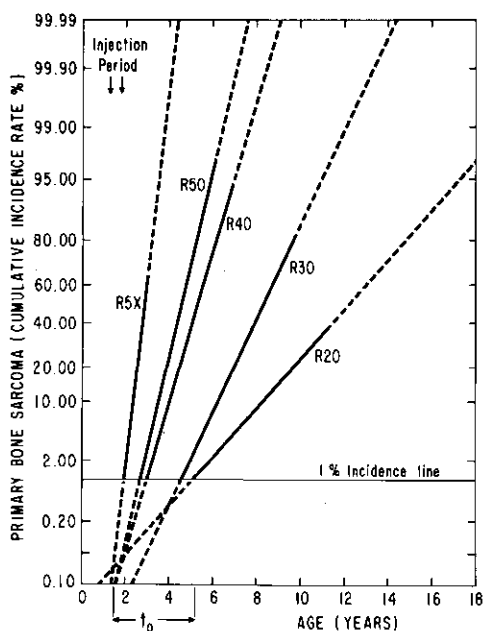


FIG.3b. Cumulative incidence of primary bone sarcomas in beagles injected with ^{226}Ra plotted on a normal probability scale as a function of age.

the combination of the 2-month and 4-month age groups. The dotted lines are suggested extrapolation to higher incidences of PBS. Age-specific cumulative incidence rate of primary sarcomas shows a normal distribution with respect to both age and ^{226}Ra dose (Figs 3b, 2). Figure 3a gives the cumulative incidence of PBS induced by ^{226}Ra plotted on a normal probability scale. The parameters for the probability density functions in years are: $\bar{T} = 2.4$, $\sigma = 0.55$ at R5X; $\bar{T} = 4.5$, $\sigma = 0.6$ at R50; $\bar{T} = 5.3$, $\sigma = 0.75$ at R40; $\bar{T} = 8.4$, $\sigma = 1.15$ at R30; and $\bar{T} = 12.3$, $\sigma = 1.25$ years at R20.

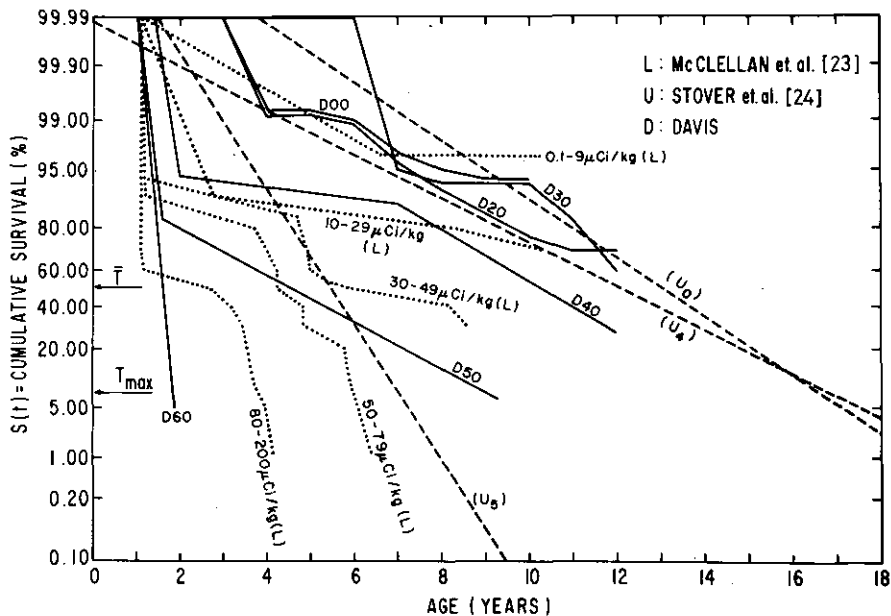


FIG.4. Age-specific cumulative survival, $S(t)$, for $^{90}\text{Sr} + ^{90}\text{Y}$ -labeled beagles plotted on a normally distributed probability scale as a function of age. Curves designated D are our data (see Table II for dose levels) for strontium ingestion dogs; L are data reported by McClellan et al. [23] for inhalation of soluble ^{90}Sr Cl_2 and U are for single injection of ^{90}Sr citrate [24]. U_0 , U_4 and U_5 are control, $32.7 \mu\text{Ci } ^{90}\text{Sr} + ^{90}\text{Y}/\text{kg}$, and $97.9 \mu\text{Ci } ^{90}\text{Sr} + ^{90}\text{Y}/\text{kg}$ respectively. T and T_{max} are median survival age ($S = 50\%$) and practical survival age ($S = 8\%$).

Figure 2 shows probability of incidence of PBS as a function of the time-integrated dose. The rate of increase in PBS with increase in dose is larger at lower dose levels.

SURVIVAL

Cumulative survival rates for our $^{90}\text{Sr} + ^{90}\text{Y}$ - and ^{226}Ra -treated beagles were plotted on a normal probability distribution scale in Figures 4 and 5. Survival of a group of beagles extrapolated to zero population for estimation of life-span depends on the last few survivors. Survival (S) data for control dogs was plotted on linear-linear graph paper and two points on this curve, at 50% and 20% survival, were connected by a straight line for extrapolation to zero. The time at this extrapolated zero population corresponds to 92% mortality assuming a normal distribution of the population. The age at 92% mortality, practical survival age, was chosen as a basis for comparison between different groups of beagles. (See Table II.)

Figure 6 gives practical survival age (T_{max}) estimated for our beagles as a function of dose for $^{90}\text{Sr} + ^{90}\text{Y}$ - and ^{226}Ra -labeled skeleton. Practical survival age decreased with increasing time-integrated dose for both $^{90}\text{Sr} + ^{90}\text{Y}$ - and ^{226}Ra -labeled beagles. Radiation-induced % lifespan shortening (Δ), i.e. the difference between control $(T_{\text{max}})^C$ and irradiated (T_{max}) and normalized to $(T_{\text{max}})^C$ is also given in Figure 6 as a function of dose. Life-

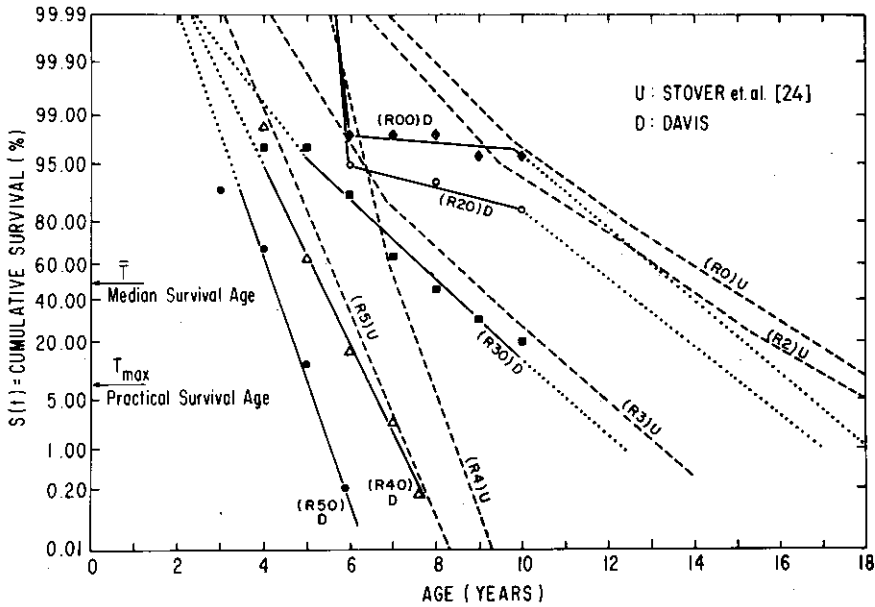


FIG.5. Age-specific cumulative survival, $S(t)$, for ^{226}Ra -injected beagles plotted on a normally distributed probability scale as a function of age. Curves designated D are our data; U are from Stover et al. [24]. Dose levels are given in Table I (D) and Fig.4 (U).

TABLE II. ^{90}Sr INGESTION DOSE LEVELS IN BEAGLES BY DAILY FEEDING FROM MIDGESTATION TO 18 MONTHS OF AGE (BB_{max} is maximum body burden at 18 months of age.)

Treatment Code	^{90}Sr ($\mu\text{Ci/g}$ dietary Ca)	Avg. Ingested/day/ (μCi)	Multiple of D10 level	(BB) max (μCi)	Number of Dogs
D00	0.000	0.00	0	0.00	79
D05	0.007	0.03	0.33	0.25	75
D10	0.021	0.08	1	0.9	42
D20	0.123	0.5	6	5.0	64
D30	0.37	1.5	18	16.3	70
D40	1.11	4	54	45.5	56
D50	3.33	12	162	121.5	47
D60	10.00	36	486	355.0	19

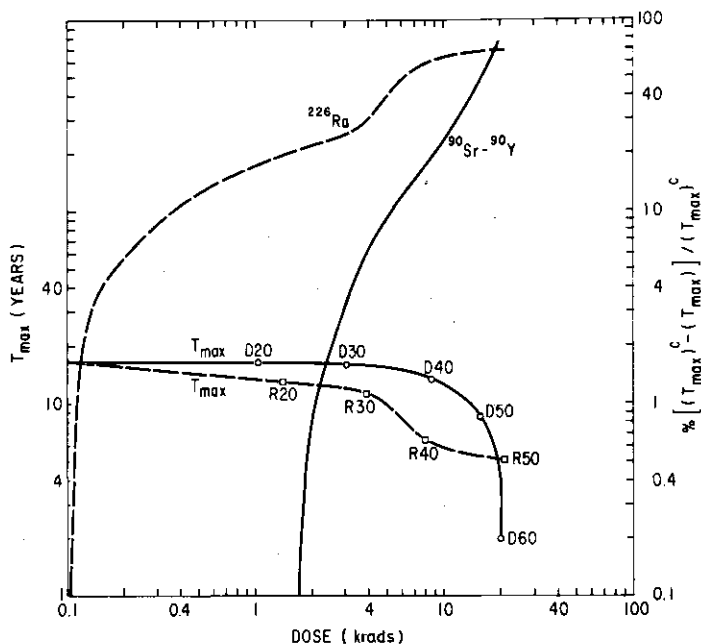


FIG.6. Practical survival age (T_{\max}) and lifespan shortening (Δ) as a function of dose integrated to (T_{\max}) for $^{90}\text{Sr} + ^{90}\text{Y}$ and ^{226}Ra -treated beagles.

span shortening increased with dose of $^{90}\text{Sr} + ^{90}\text{Y}$ from $\Delta \approx 0$ at 1.7 krad to $\Delta = 75\%$ at 20 krad. A dose of 1.7 krad is accumulated from a dose level D20, daily ingestion of $0.5 \mu\text{Ci } ^{90}\text{Sr} + ^{90}\text{Y}/\text{day}$. The life-shortening curve for ^{226}Ra group shows a biomodal structure. At 20 krad, $^{90}\text{Sr} + ^{90}\text{Y}$ and ^{226}Ra are equally effective in reducing practical survival age.

Figure 7 gives practical survival age (T_{\max}) as a function of the maximum dose rate \dot{D}_{\max} for $^{90}\text{Sr} + ^{90}\text{Y}$ - and ^{226}Ra -labeled skeletons. The maximum dose rates were evidenced at 1.5 years of age for both $^{90}\text{Sr} + ^{90}\text{Y}$ and ^{226}Ra . The practical survival age decreased with increasing maximum dose rate. Life-span shortening (Δ) increased with \dot{D}_{\max} monotonically for both $^{90}\text{Sr} + ^{90}\text{Y}$ - and ^{226}Ra -labeled dogs. At a ^{226}Ra dose of 100 rad accumulated over 16.5 years from an injected activity of $0.2 \mu\text{Ci}$ resulted in $\Delta \approx 0$.

The R5X group survived to $T_{\max} \approx 3$ years, whereas the R50 group survived to 5.2 years. This indicates a differential life-span shortening of about 2 years in beagles injected at 2 or 4 months of age relative to those injected as young adults.

DISCUSSION

Spontaneous primary bone sarcomas are relatively uncommon in dogs [18], especially in beagles [19]. These spontaneous tumors originate in the metaphyses of the long bones of the limbs mostly in the proximal humerus and distal radius.

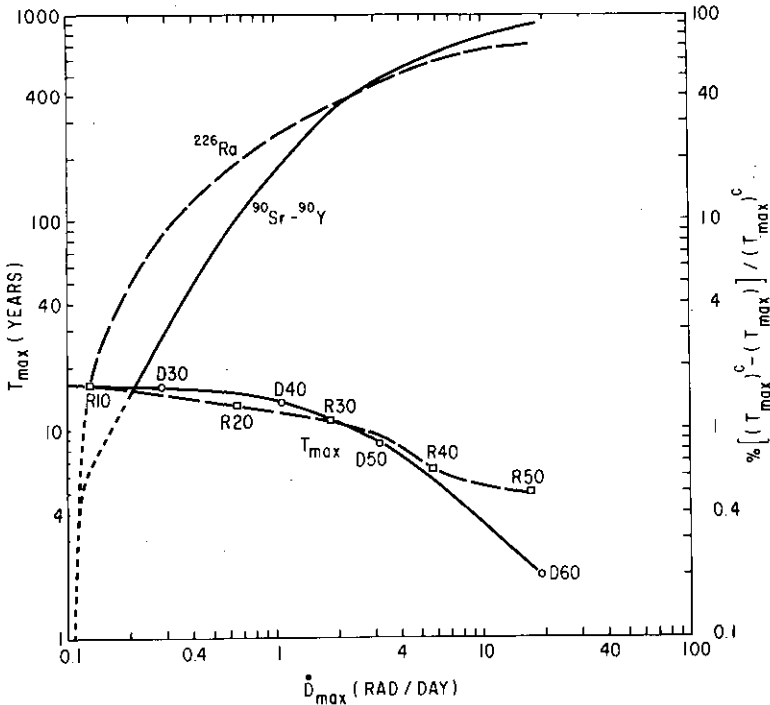


FIG.7. Practical survival age (T_{\max}) and lifespan shortening (Δ) as a function of the maximum dose rate (\dot{D}_{\max}) for $^{90}\text{Sr} + ^{90}\text{Y}$ and ^{226}Ra -treated beagles.

Probability of occurrence of radiation-induced PBS is a function of time post-injection, age, and injection level. The age-specific cumulative incidence rate of primary bone sarcomas (CPBS) is higher at higher $^{90}\text{Sr} + ^{90}\text{Y}$ levels of injection. There is a longer delay for observation of PBS at lower levels (e.g. D40), but the longer accumulation of dose makes this radiation level more effective in induction of PBS per rad than the higher (D60) level which had a 9 times higher dose rate. The importance of time factor in radiation toxicity was recognized by Brues et al. [20] and was the basis for the concept of cumulative rad-year [21].

The R50 and R40 dose levels reached a 100% age-specific cumulative incidence rate of primary bone (CPBS) (Fig. 2). Maximum estimated incidence of primary bone sarcomas (PBS) for R30 level is 90% and for R20 is 40% at ages 12 and 15 years, respectively. The estimated doses at the time of maximum incidence of PBS is 30, 9.5, 4 and 1.2 krad, respectively, for R50, R40, R30 and R20 levels. Comparison of ^{226}Ra and $^{90}\text{Sr} + ^{90}\text{Y}$ for incidence of primary bone sarcomas shows that on the basis of age (Figs 1a, b and 3a, b) D60 and D50 are similar to R5X and R30, respectively. On the basis of time-integrated dose (Fig. 2), incidence of PBS for D60 level is comparable to R50, and the R20 is midway between D50 and D40. The ages to observe a 1% PBS incidence (t_0) are estimated to be 9.4 year (D40), 5.1 year (D50) and 1.5 year (D60) for $^{90}\text{Sr} + ^{90}\text{Y}$ dogs. For ^{226}Ra dogs, estimated time between the

1% incidence and final injection of ^{226}Ra are 3.8 year (R20), 3.3 year (R30), 1.7 year (R40) and 1.3 year (R50). At R5X level, $t_0 = 0.6$ years.

The average time-integrated skeletal doses of $^{90}\text{Sr} + ^{90}\text{Y}$ to induce a 1% incidence of PBS (D_d) were estimated (Fig. 2) as 0.6 krad at D40 level, 1.2 krad at D50 level, and 12.8 krad at D60 level. Similarly, D_d for radium dogs were 0.80 krad at R20, 1.8 krad at R30, 4.3 krad at R40, and 12.8 krad at R50 level.

Distribution of $^{90}\text{Sr} + ^{90}\text{Y}$ -induced PBS in our dogs was 32% in head, 9% in vertebral column, 27% in pectoral limbs and 32% in pelvic limbs [22]. Distribution of ^{226}Ra -induced PBS was 10% in head, 19% in vertebral column, 1% in rib cage, 34% pectoral limbs, and 35% in pelvic limbs [1]. Casarett [22] observed that the most cancellous fractions of the skeleton are the most common sites of involvement in radiation-induced skeletal tumors. With $^{90}\text{Sr} + ^{90}\text{Y}$ ingestion, skeletal matrix is uniformly labeled during the 1.5 years of radioactivity ingestion. However, after the cessation of the $^{90}\text{Sr} + ^{90}\text{Y}$ feeding, trabecular sites lose their activity at a rate 25 times faster than do those in the cortex. Skeletal uptake in ^{226}Ra -injected beagles is mostly by appositional growth and focal areas of remodeling. The activity appears mostly on the periosteal and the endosteal surfaces [13]. Thus the disparity in the principal sites of tumor incidence between ^{90}Sr and ^{226}Ra may be related to the radiation history of these regions in addition to the cellular turnover and repair.

The observed life-span shortening among irradiated dogs was attributed mostly to radiation-induced tumors. Figures 6 and 7 show that in this study the practical survival age (T_{\max}) is relatively uninfluenced by irradiation from $^{90}\text{Sr} + ^{90}\text{Y}$ to a level less than D40. At higher ingestion levels radiation-induced life shortening increased rapidly with dose. A beagle on a D60 level survived 20% of its normal lifespan in comparison with a D30 level beagle in which survival is estimated to be 97% of its normal lifespan, essentially that of the control dog. ^{226}Ra -labeled beagles show life shortening at the R20 level, at which life has already been reduced by 20% at a dose of 1.5 krad. However, at 20 krad both $^{90}\text{Sr} + ^{90}\text{Y}$ and ^{226}Ra are equally effective in reducing the natural beagle lifespan.

A maximum dose rate of 1 rad/day (Fig. 7) reduced T_{\max}^C by 17% for $^{90}\text{Sr} + ^{90}\text{Y}$ exposure and 25% for ^{226}Ra exposure. However, a dose rate of 6 rad/day from ingested ^{90}Sr (D-series) is more lethal than that from injected ^{226}Ra .

One difference between the lifespan shortening effect of $^{90}\text{Sr} + ^{90}\text{Y}$ and ^{226}Ra is the comparative risk between primary bone sarcomas and myeloproliferative disorders. For $D_{\max} > 2.5$ rad/day, $^{90}\text{Sr} + ^{90}\text{Y}$ reduced average lifespan by removing beagles from test population with osteosarcoma and/or myeloproliferative disease. Of all dogs on D60 level, about 20% were afflicted with myeloproliferative disease in comparison with about 30% at D50 level and about 12% at D40 level [11-12]. Our final estimate for cumulative incidence rates are 44% and 50% for D60 and D50 levels, respectively, and the predicted maximum cumulative incidence rate (MCIR) for D60 level is 88%. The reason for the difference between the MCIR = 44% observed and the 88% predicted seems to be from early deaths due to competition from primary bone sarcomas. In contrast, ^{226}Ra -labeled dogs are mostly afflicted with bone neoplasms such as osteosarcomas, a few chondrosarcomas, and fibrosarcomas.

Figures 4 and 5 compare the cumulative survival rate, $S(t)$, for our beagles with those reported by McClellan et al. [23] and Stover et al. [24]. The linear curve on Figure 4 indicates a normally distributed survival function for $^{90}\text{Sr} + ^{90}\text{Y}$ -labeled dogs. At D60 level, $S(t)$ shows a median survival age ($S = 50\%$) of $\bar{T} = 1.5$ years and a $T_{\max} = 1.9$ years. No other dose level exhibits single normal distribution population for survival although the curves may represent a combination of two or more normally distributed populations of risks. Choice of \bar{T} as survival age parameter for comparison with dose leads toward biases in favor of a disease with a shorter delay period and a subpopulation that is more sensitive to radiation. Thus, using \bar{T} as the sole estimator for survival when there is more than one mode (subpopulation or risk) could lead to erroneous conclusions.

Cumulative survival rates for 142 control beagles were calculated using a life-table method [25] from the data reported by the Radiobiology Laboratory, University of Utah [26]. The composite data indicated $\bar{T} = 13.6$ and $T_{\max} = 16.5$ years. Similar calculations for our older control beagles indicates a close similarity with respect to survival distribution between these two beagle populations. Figure 7 gives cumulative survival rate for ^{226}Ra -injected beagles plotted as a function of age [24]. The cumulative survival rates are normally distributed, probably because the risk from radium is mostly from primary bone sarcomas and is less affected by competitive risks from other diseases. The practical survival age, T_{\max} , estimated from our data are 5.1, 6.4, 11.5 and 14 years, respectively, for R50, R40, R30 and R20. T_{\max} estimated for beagles administered a comparable dose of radium but in a single injection is 5.2 (R5), 6.3 (R4), 9.6 (R3), 15.7 (R2), 16.5 years (R0 - R1.7). The estimated difference for T_{\max} of the R20 and the R2 groups is 1.7 years in favor of the singly injected dogs. It is of interest to note that the youngest R2 (single injection) dog succumbing to primary bone sarcomas was 10.4 years old, whereas the R20 (8-injections) counterpart was 4.5 years old; whereas the pattern for groups injected at higher dosages was the same. Radiation-induced primary bone sarcomas and reduced longevity from $^{90}\text{Sr} + ^{90}\text{Y}$ and ^{226}Ra body burden are dependent, in addition, on age at administration of the radioactivities and mode of administration, on interaction of dose rate, dose, and time with a nonlinear dynamic organ system, the skeleton.

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DISCUSSION

E.J. AINSWORTH: I am seeking interrelationships between external and internal irradiation data. It seems to me that your results with ^{90}Sr and ^{226}Ra may be suitable for evaluation of the RBE for life shortening as a function of accumulated or total dose. What do you think?

M.H. MOMENI: The survival function for beagles with $^{90}\text{Sr} + ^{90}\text{Y}$ and ^{226}Ra is not parallel, and thus the RBE is not unique and dependent on the magnitude of the effect. However, RBE increases from a small value at low dose and maximum dose rates to a value larger than unity at high levels of radiation dose. This may be a reflection of LET as well as the range of the two types of radiations (α and β) irradiating different cell types. The response upon exposure to ^{90}Sr radiation is from both osteosarcomas and haematopoietic syndrome.

E.J. AINSWORTH: I have a comment on what Dr. Mays has called a reversed dose-rate effect. As you know, we have observed a similar phenomenon with external exposure to fission spectrum neutrons — we have termed it "enhancement". My point is that the enhancement phenomenon is not restricted to internal emitters of high LET radiations and bone tumors. Our results show enhancement of life shortening and an earlier appearance of, at least, pulmonary tumours. Current work with neutrons will tell us about the dose range over which the enhancement occurs.

If we look broadly at the data from internal and external exposure experiments, it may be possible to establish a generalization regarding the relative effects and risks associated with single or protracted exposures to high LET radiations.

W.H. ELLETT: The increased radiocarcinogenicity at low dose and dose rates may not be confined to high LET radiations, as implied by Dr. Ainsworth. Did your data not indicate, for both ^{226}Ra and ^{90}Sr (a low-LET β emitter), an increase in the cancer incidence per rad as the dose and dose rate decreased?

M.H. MOMENI: Yes, the data indicate a smaller D_0 or delay dose as the maximum dose rate is decreased. This increase in radiation efficiency with decrease in the maximum dose rate shows the effect of time factor to allow full expression of a radiation dose.

RESPONSE OF MOUSE FOETUS TO RADIATION FROM $\text{Na } ^{99\text{m}}\text{TcO}_4^*$

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Abstract

RESPONSE OF MOUSE FOETUS TO RADIATION FROM $\text{Na } ^{99\text{m}}\text{TcO}_4$.

The element technetium has recently assumed ecological importance as a source of low-level radiation, with the use of $^{99\text{m}}\text{Tc}$ in nuclear medicine and production of ^{99}Tc during generation of electricity by nuclear reaction. When technetium is introduced as pertechnetate into the bloodstream of pregnant females, it is transported across the placental barrier to the foetus, where a portion appears to be incorporated into biomolecules. When combined as biomolecules, radionuclides that decay by electron capture or isomeric transition show a lethality greater than that predicted in cell cultures and radiation therapy. The decay of $^{99\text{m}}\text{Tc}$ by isomeric transition, together with the above considerations, places a high priority on the investigation of its radiation effects due to clinical doses of up to 25 mCi. Female mice were given daily intravenous injections of 0, 5, 50 and 500 μCi of $^{99\text{m}}\text{Tc}$ as pertechnetate in isotonic saline throughout gestation, gestation and lactation, or lactation. At two months of age, the progeny were mated with randomly selected litter mates to produce a second generation; the process was repeated with their progeny for production of the third generation. Only the first generation was irradiated. An injection of 5 μCi /mouse and 10 mCi/human both approximately equal 0.2 $\mu\text{Ci/g}$ of body weight; however, the mouse foetus receives an estimated radiation dose of 0.05 rad, and the human 1 rad. Throughout the gestation period, a mouse at the 5 μCi level receives about 100 μCi of $^{99\text{m}}\text{Tc}$, resulting in approximately 1 rad to the foetus. Significant reduction of body weight was noted in all experimental groups compared with weights of control animals. The greatest effects were seen in the progeny of mothers treated during gestation only. A tendency toward recovery seen in the third generation may possibly be explained by elimination of some animals from the genetic pool. These preliminary results reinforce the existing concern about use of $^{99\text{m}}\text{Tc}$ -pertechnetate in pregnant or potentially pregnant subjects.

INTRODUCTION

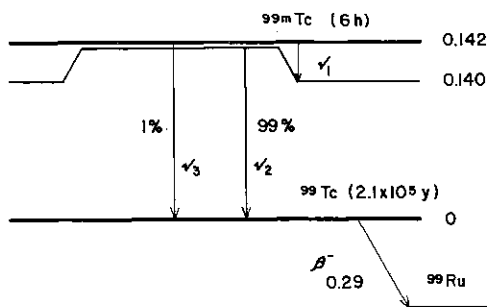
A new ecological source of low-level radiation has appeared within the last decade. The element technetium has attained biological importance due to extensive use of isotope $^{99\text{m}}\text{Tc}$ in medicine and to release of isotope ^{99}Tc into the biosphere from nuclear weapons testing and from production of electricity by nuclear reaction.

Between one and five percent of the world's population is estimated to be examined diagnostically each year with $^{99\text{m}}\text{Tc}$ as sodium pertechnetate. Even larger quantities of $^{99\text{m}}\text{Tc}$ may be administered in the form of other radio-pharmaceuticals. Since 1963, increasingly greater amounts of $^{99\text{m}}\text{Tc}$ ($t_{1/2}$, 6h) have been used medically each year, so that as much as 70 mCi daily was found in a 1973 study of Cincinnati, Ohio, sewage [1], presumably excreted by patients.

Terrestrial presence of technetium-99 was first recognized in 1961 when the element was isolated from uranium ore. The existence of technetium had been proven in the 1940's by the production of short-lived isotopes of the element

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FIG. 1. Decay scheme for ^{99m}Tc and ^{99}Tc .

from nuclear bombardment of molybdenum [2]. Nonradioactive isotopes of technetium have never been isolated and apparently cannot exist [3]. By 1962, an estimated 50 Ci of ^{99}Tc had been produced from atmospheric testing of nuclear weapons [4]. Between 1963 and 1980, an additional 170 000 Ci (10 000 kg) will have accumulated from the production of electrical energy [5]. Technetium may be present in the atmosphere as the volatile heptoxide [6], which may be absorbed from the lungs [7]. It may also be present as pertechnetate, readily absorbed after ingestion and incorporated into unidentified compounds by plants [8] before consumption by animals.

These two radioisotopes, ^{99m}Tc and ^{99}Tc , have the decay characteristics diagrammed in Fig. 1, and the energy released by transitions in ^{99m}Tc are listed in TABLE I. It is obvious that the low-energy radiations from both of these isotopes will result in high-intensity radiation fields immediately surrounding the atom. Decay may be highly destructive to a cell or biomolecule into which the atom is incorporated. This may be especially true for the decay of ^{99m}Tc which takes place partially by isomeric transition. This process, together with electron capture, has been shown to be particularly disruptive [9], and to produce radiation effects greater than those predicted from the amount of energy released, both in cell cultures [10] and in radiation therapy [11]. Previously unpublished studies from this laboratory suggest that ^{99m}Tc , when administered as $\text{Na}^{99m}\text{TcO}_4$, is combined into biomolecules in the mammalian thyroid and fetus. In addition to radiation damage, chemical toxicity for animals and plants from long-lived ^{99}Tc may be ecologically important [8]. These two, radiation and chemical toxicity, cannot be studied independently for technetium since no stable isotope exists.

As an initial attack on the problem, we chose to study the biological distribution and radiation effects of ^{99m}Tc in the mammalian fetus. The fetus was chosen because technetium is apparently bound into biomolecules and because the question of possible damage from ^{99m}Tc to genetic material (essential for survival as man now comprehends it) is unanswered. Technetium-99m as pertechnetate was chosen because a significant segment of the population is exposed to this radiopharmaceutical; because the distribution patterns found with virtually carrier-free amounts of technetium are essential for further studies to assess its chemical toxicity; and because the gamma radiation of ^{99m}Tc is more readily assayed than the beta radiation from ^{99}Tc .

METHODS AND RESULTS

The concentration of ^{99m}Tc in the feto-placental unit of the mouse versus the day of gestation was determined by intravenous injection of 0.1 ml $\text{Na}^{99m}\text{TcO}_4$ in 0.9 % NaCl into three pregnant CF-1 mice, sacrifice after 30 min, and assay of the excised feto-placental unit. Such a study was made on each day of gestation. Day one of gestation was determined on finding of a vaginal

Table I
Radiations and Energy Released
by Decay of ^{99m}Tc *

Radiation		Mean Number/ Disinte- gration	Mean Energy/ Par- ticle	Equi- librium Dose Constant
	n_i		\bar{E}_i (MeV)	(g·rad/ $\mu\text{Ci}\cdot\text{h}$)
Gamma	1	0.0000	0.0021	0.0000
M Int Con Elect		0.9860	0.0016	0.0035
Gamma	2	0.8787	0.1405	0.2630
K Int Con Elect		0.0913	0.1194	0.0232
L Int Con Elect		0.0118	0.1377	0.0034
M Int Con Elect		0.0039	0.1400	0.0011
Gamma	3	0.0003	0.1426	0.0001
K Int Con Elect		0.0088	0.1215	0.0022
L Int Con Elect		0.0035	0.1398	0.0010
M Int Con Elect		0.0011	0.1422	0.0003
K Alpha-1 X-ray		0.0441	0.0183	0.0017
K Alpha-2 X-ray		0.0221	0.0182	0.0008
K Beta-1 X-ray		0.0105	0.0206	0.0004
KLL Auger Elect		0.0152	0.0154	0.0005
KLX Auger Elect		0.0055	0.0178	0.0002
LMM Auger Elect		0.1093	0.0019	0.0004
MXV Auger Elect		1.2359	0.0004	0.0011

*Taken from reference [12].

plug by visual inspection the day following overnight caging with a male proven to be fertile. The female was then immediately separated from the male. Results from this experiment, represented graphically in Fig. 2, show a sharp increase in total uptake by the feto-placental unit from about 5 % at the end of the second week to almost 50 % of the injected ^{99m}Tc at term (21 days). As the fetus increases in weight, the concentration (% per g) decreases, so that the radiation dose averaged over the total fetal weight also decreases with gestatory age.

Incorporation of ^{99m}Tc into biomolecules was demonstrated indirectly through study of its concentration by, and release from, tissue in the presence of perchlorate ion, which acts only on pertechnetate, not on organic combinations with technetium. One day before expected termination of pregnancy, mice were injected with $\text{Na}^{99m}\text{TcO}_4$ alone, at 15 min after, and 30 min or 1 h before NaClO_4 . The latter was administered intravenously, 15 mg in 0.1 ml solution, an amount required to produce maximum inhibition of localization in the mouse thyroid gland and gastric mucosa. All animals were sacrificed 90 min after the ^{99m}Tc had been given intravenously. The results, summarized in TABLE II, indicate considerable binding of Tc in the placenta and fetus since the concentration is not changed when perchlorate is given, although discharge occurs from the thyroid gland and gastric mucosa.

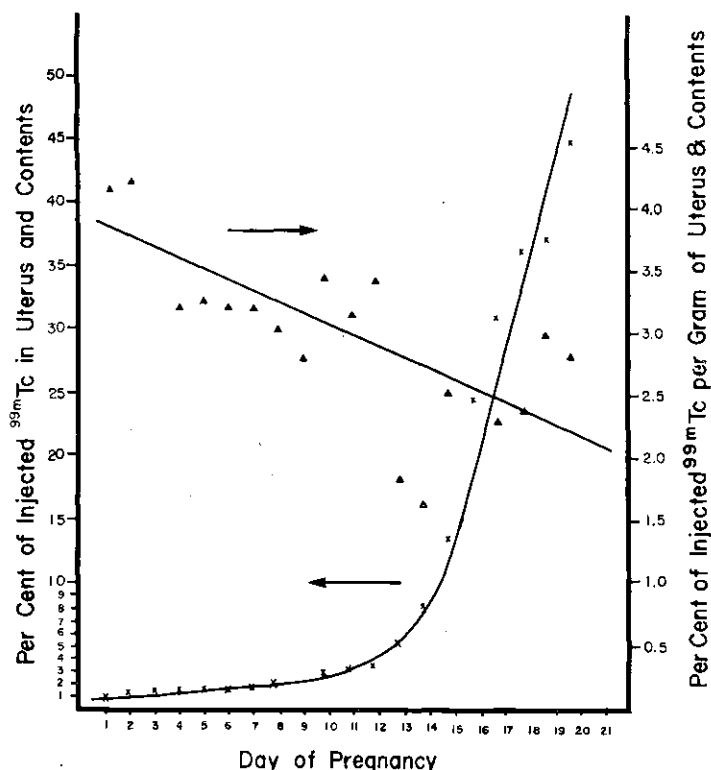


FIG. 2. ^{99m}Tc in the mouse uterus with contents 30 min after i. v. administration of $\text{Na}^{99m}\text{TcO}_4$ to the mother.

The distribution of ^{99m}Tc in the mouse fetus was documented by intravenous administration of $\text{Na}^{99m}\text{TcO}_4$ to mice on the day before expected spontaneous delivery, followed by dissection and assay of the fetal and maternal organs at 15, 30, 60 and 120 min. The concentrations of ^{99m}Tc in selected fetal and maternal organs, represented graphically in Fig. 3, differ markedly for the thyroid gland where the maternal concentration is several times greater, and for the spleen, liver, femur and blood where the fetal concentration is more. The actual concentration of ^{99m}Tc in the fetal kidney is about twice the maternal concentration at all time intervals, but the kidney/blood ratios are comparable, thus suggesting operation of a similar mechanism in the fetus and mother. These data suggest that ^{99m}Tc is presented to the fetus in a chemical combination that is not pertechnetate; indicate absorbed doses for fetal organs different from the maternal; and, together with results from the perchlorate experiments, imply that Tc may be converted to a non-pertechnetate form in the placenta before transfer to the fetus.

The effect of radiation from ^{99m}Tc on the fetus, the neonate and their progeny was assessed by daily intravenous injection of the mother with 0, 5, 50 or 500 μCi of $\text{Na}^{99m}\text{TcO}_4$, contained in 0.1 ml of 0.9 % NaCl, throughout gestation, gestation and lactation, or lactation, according to the scheme diagrammed in Fig. 4. Only the animals in group 1 were irradiated, in utero or as neonates, or both. The group-1 progeny of treated mothers were randomly mated with litter mates at 2 months of age to produce group 2, which were similarly

Table II
Effect of NaClO_4 on Uptake by the Feto-Placental Unit of
 $^{99\text{m}}\text{Tc}$ Administered Intravenously as $\text{Na}^{99\text{m}}\text{TcO}_4$

Treatment	% of Injected $^{99\text{m}}\text{Tc/g}^*$			
	Placenta	Fetus	Amniotic Fluid	Maternal Thyroid
$\text{Na}^{99\text{m}}\text{TcO}_4$	2.3 $\pm 0.02^\dagger$	2.3 ± 0.016	0.55 ± 0.02	100 ± 2.4
NaClO_4 (i.v.) 15 min before $\text{Na}^{99\text{m}}\text{TcO}_4$	1.9 ± 0.008	0.98 ± 0.001	1.0 ± 0.01	0
$\text{Na}^{99\text{m}}\text{TcO}_4$ 30 min before NaClO_4 (i.v.)	2.4 ± 0.02	2.2 ± 0.009	1.7 ± 0.02	11 ± 1.0
$\text{Na}^{99\text{m}}\text{TcO}_4$ 1 h before NaClO_4 (i.v.)	2.5 ± 0.01	2.0 ± 0.018	0.90 ± 0.03	7.8 ± 0.39

* 90 min after $\text{Na}^{99\text{m}}\text{TcO}_4$.

† Average for 3 mice.

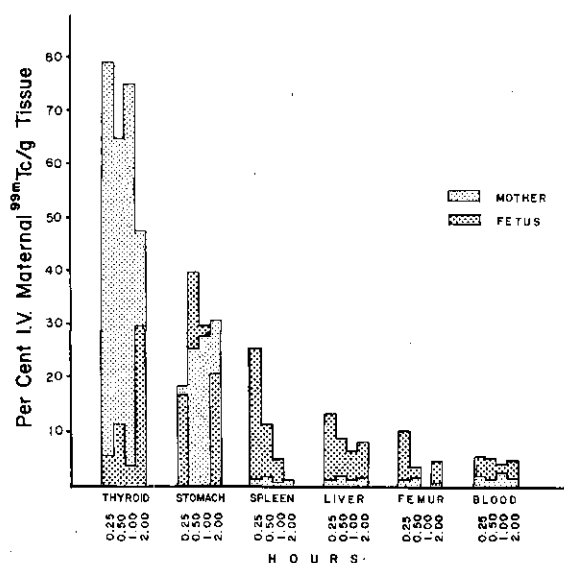


FIG. 3. Concentration of $^{99\text{m}}\text{Tc}$ in fetal and maternal organs after i. v. administration of $\text{Na}^{99\text{m}}\text{TcO}_4$ to the mother.

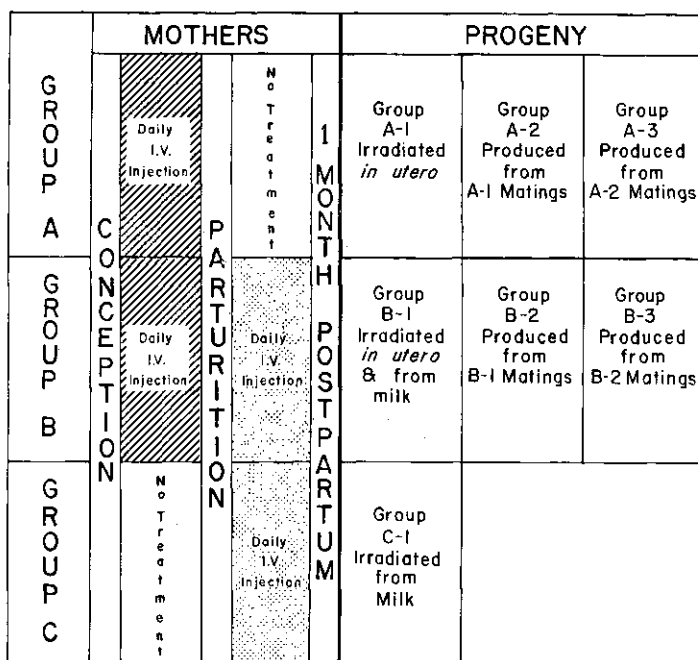


FIG. 4. Diagram of experimental procedure.

mated to yield groups 3. An injection of 5 μCi per mouse and one of 10 mCi per human are both approximately equal to 0.2 $\mu\text{Ci/g}$ of body weight. However, because of differences in absorbed fractions, the mouse fetus receives an estimated radiation dose of 0.05 rad, whereas the human fetus receives 1 rad. A mouse on the 5 μCi daily dose receives about 100 μCi throughout the gestation period, resulting in approximately 1 rad to the fetus.

Birth weights of the fetuses, as indicated by differences in maternal weights on the day before and the day after delivery, were not affected by exposure to *in utero* radiation from ^{99m}Tc (TABLE III). Significant differences from controls were noted, however, at 1 and 2 months of age. Mean weights at 1 and 2 months for animals in each group and the statistically significant deviation from the control group for each are shown in TABLE IV. *In utero* irradiation (group A) resulted in highly significant reduction of weight for the progeny of mothers receiving 50 or 500 μCi of ^{99m}Tc daily throughout gestation, and the effect carried through the succeeding two generations. A tendency toward recovery noted in the third generation may represent loss of some animals from the genetic pool.

Exposure of mice to irradiation throughout gestation and during lactation (group B) also produced animals weighing less than their controls in the 50 and 500 μCi groups, with some recovery likewise noted in the next two generations. The significantly different weights of second-generation animals from mothers given 5 μCi , not seen in the first and third generations, is most puzzling.

Exposure of mice to ^{99m}Tc in milk from their lactating mothers (group C) produced a significant weight reduction only in the females of the 500 μCi group. A more striking effect was the appearance of hairlessness in 4 of the 10 litters born to the 500 μCi mothers. Of the 25 survivors in these four

Table III
Weights of Mice Dams Receiving $\text{Na}^{99\text{m}}\text{TcO}_4$
Throughout Gestation

Weighed	Weight in g \pm S.D. (N)			
	NaCl	5 μ Ci	50 μ Ci	500 μ Ci
Day of conception	27.9 \pm 2.8 (43)	27.7 \pm 2.9 (46)	27.2 \pm 3.0 (42)	27.7 \pm 2.6 (41)
One day before delivery	52.1 \pm 5.1 (43)	51.4 \pm 6.7 (46)	51.7 \pm 6.0 (42)	51.3 \pm 5.2 (41)
One day after delivery	34.1 \pm 4.4 (42)	33.8 \pm 3.8 (45)	33.2 \pm 4.0 (42)	33.4 \pm 3.1 (41)

litters at one month, 14 mice, 12 of them females, were hairless. No pregnancies resulted from matings of the progeny of the mothers treated during lactation, either with litter mates or with mates having no history of radiation.

A comparison of the weights of first- with second-generation progeny was made by determining the mean \pm S.D. for each group (data shown in TABLE IV), and then, finding the ratio between the experimental group and its control, i.e.,

$$R = \frac{M_{\text{control}}}{M_{\text{experimental}}} \pm \frac{M_{\text{control}}}{M_{\text{experimental}}} \sqrt{\frac{\sigma^2/N}{M_{\text{control}}^2} + \left(\frac{\sigma^2/N}{M_{\text{experimental}}^2}\right)}$$

Two independent groups were then compared by use of the ratios:

$$\frac{R_1 - R_2}{\sqrt{\sigma_1^2 + \sigma_2^2}}$$

Some results of such analyses are shown in TABLE V.

In no groups did the weights of A-1 males differ significantly from A-1 females, nor did A-2 males differ from A-2 females, probably indicating that no sex difference in radiation effect exists. Results were similar for corresponding B groups, except for a slight tendency toward heavier females. B-1 animals are comparatively heavier than A-1, and the tendency is more pronounced in the B-2 and A-2 groups.

Comparison of radiation effects on the fetus due to $^{99\text{m}}\text{Tc}$ given intravenously to the mother with the effects of other radionuclides or other forms of radiation is most difficult because of differences in tissue distributions, decay modes, and uniformity of radiation fields. Such analyses probably should be attempted after more data have been accumulated in these areas.

TABLE IV
WEIGHTS OF SURVIVING MICE AT 1 AND 2 MONTHS

GROUP	GENERATION	MONTH	Weight (g) \pm S.D. [N]							
			NaCl Controls ¹		5 μ Ci Na ^{99m} TcO ₄ ¹		50 μ Ci Na ^{99m} TcO ₄ ¹		500 μ Ci Na ^{99m} TcO ₄ ¹	
			Male	Female	Male	Female	Male	Female	Male	Female
A	1	1	16.7 \pm 2.9[69]	13.7 \pm 2.5[92]	16.8 \pm 2.8[52]	13.7 \pm 2.9[77]	13.9 \pm 3.8[75]	11.3 \pm 3.4[812]	12.5 \pm 3.2[63]	9.8 \pm 3.6[67]
		2	26.5 \pm 2.4[64]	22.5 \pm 3.5[75]	26.4 \pm 3.3[51]	22.5 \pm 3.2[69]	25.9 \pm 4.0[68]	21.2 \pm 5.2[78]	24.0 \pm 2.8[50]	19.9 \pm 3.5[55]
	2	1	16.4 \pm 2.1[48]	13.6 \pm 2.8[55]	14.5 \pm 2.3[48]	11.9 \pm 2.4[60]	14.6 \pm 1.4[40]	11.7 \pm 2.2[71]	12.7 \pm 1.2[3]	9.8 \pm 2.8[8]
		2	25.0 \pm 4.0[48]	21.6 \pm 4.1[55]	21.1 \pm 3.0[48]	19.0 \pm 3.2[60]	21.1 \pm 2.6[40]	17.8 \pm 2.3[71]	20.3 \pm 0.6[3]	17.9 \pm 1.9[7]
	3	1	14.5 \pm 3.3[35]	14.0 \pm 2.9[42]	15.4 \pm 4.2[44]	14.5 \pm 3.1[55]	14.6 \pm 4.3[43]	13.0 \pm 3.6[55]	9.5 \pm 3.6[13]	10.1 \pm 4.2[14]
		2	23.4 \pm 2.5[26]	20.7 \pm 3.3[29]	23.3 \pm 2.7[42]	21.9 \pm 2.6[45]	21.4 \pm 4.0[31]	23.2 \pm 3.2[47]	19.9 \pm 5.5[8]	19.9 \pm 5.0[11]
B	1	1	15.2 \pm 3.3[55]	13.1 \pm 3.5[48]	15.2 \pm 3.2[60]	12.9 \pm 2.6[67]	14.2 \pm 2.7[48]	11.6 \pm 2.7[49]	11.3 \pm 3.2[47]	9.2 \pm 3.0[54]
		2	26.1 \pm 2.6[53]	21.7 \pm 2.4[44]	26.6 \pm 2.5[59]	23.3 \pm 2.7[66]	25.3 \pm 2.2[47]	21.5 \pm 3.7[46]	26.3 \pm 2.6[43]	22.0 \pm 2.9[42]
	2	1	15.1 \pm 1.5[21]	12.2 \pm 1.5[31]	13.7 \pm 3.3[67]	11.2 \pm 3.1[66]	14.3 \pm 2.0[40]	11.6 \pm 2.3[50]	13.8 \pm 1.0[28]	11.7 \pm 0.7[26]
			21.2 \pm 2.1[21]	18.8 \pm 1.9[31]	20.0 \pm 2.7[61]	17.7 \pm 2.4[56]	20.6 \pm 2.2[40]	17.5 \pm 2.1[50]	19.9 \pm 2.4[28]	16.4 \pm 1.9[26]
	3	1	16.3 \pm 2.1[52]	15.4 \pm 2.2[37]	15.7 \pm 4.6[30]	14.4 \pm 3.7[29]	14.9 \pm 4.9[37]	12.3 \pm 4.2[32]	14.8 \pm 4.6[84]	13.6 \pm 3.4[57]
		2	24.4 \pm 1.8[50]	21.5 \pm 1.6[36]	24.0 \pm 2.8[24]	21.6 \pm 2.6[24]	23.6 \pm 2.1[28]	21.0 \pm 2.7[26]	22.2 \pm 3.5[71]	20.4 \pm 3.8[54]
C	1	1	18.7 \pm 2.5[41]	15.1 \pm 2.5[33]			18.6 \pm 2.8[28]	15.1 \pm 3.4[33]	18.2 \pm 2.2[23]	13.0 \pm 3.9[32]
		2	24.9 \pm 4.8[39]	20.8 \pm 2.4[31]			25.1 \pm 3.4[28]	20.9 \pm 3.5[31]	23.7 \pm 2.4[23]	18.4 \pm 3.8[29]

¹Daily dose administered i.v. to mothers of 1st generation throughout gestation.

Not significantly different from controls.

Significantly different from controls at P < 0.1 > 0.01

" " " " " P < 0.01 > 0.001

" " " " " P < 0.001



TABLE V

Confidence Levels of Comparative Weight Differences Observed
Between Various Groups of Progeny from Mothers Given
 $\text{Na}^{99\text{m}}\text{TcO}_4$ During Gestation (A) or Gestation
and Lactation (B)

Groups Compared	5 μCi		50 μCi		500 μCi	
	1 mo	2 mo	1 mo	2 mo	1 mo	2 mo
A-1M/A-1F [†]	*	*	*	*	*	*
A-2M/A-2F	*	*	*	*	*	*
A-1M/A-2M	A-1>A-2 P>0.001	A-1>A-2 P<0.001	A-1>A-2 P>0.001	A-2>A-1 P<0.001	*	A-2>A-1 P>0.001
A-1F/A-2F	A-1>A-2 P>0.001	A-1>A-2 P>0.001	*	A-2>A-1 P>0.001	*	*
B-1M/B-1F	*	F>M P>0.01	*	*	*	*
B-2M/B-2F	*	*	*	*	*	F>M P>0.01
B-1M/B-2M	B-1>B-2 P>0.01	B-1>B-2 P>0.01	*	*	B-1>B-2 P<0.001	B-2>B-1 P>0.01
B-1F/B-2F	*	B-1>B-2 P<0.001	*	*	B-1>B-2 P<0.001	B-2>B-1 P<0.001
A-1M/B-1M	*	*	B-1>A-1 P>0.001		*	B-1>A-1 P>0.001
A-1F/B-1F	*	B-1>A-1 P>0.01	*		*	B-1>A-1 P<0.001
A-2M/B-2M	*	B-2>A-2 P>0.001	*	B-2>A-2 P<0.001	B-2>A-2 P>0.01	B-2>A-2 P<0.001
A-2F/B-2F	*	*	B-2>A-2 P<0.01	B-2>A-2 P<0.01	B-2>A-2	*

[†] M = male, F = female.

* = not significantly different.

DISCUSSION AND CONCLUSIONS

This work is regarded as a series of preliminary observations requiring substantiation in other mammals for the tissue localization studies, and by other experiments carefully designed to eliminate biases for the radiation effects studies.

The investigations described appear to indicate:

- 1) Binding of Tc in the placenta, with a subsequent tissue distribution in the fetus different from the maternal tissue localization of pertechnetate.

- 2) No reduction in the birth weight of mice continuously exposed in utero to ^{99m}Tc .
- 3) A reduction of body weight in maturing mice exposed in utero to ^{99m}Tc that is transmitted to the second and third generations.
- 4) Appearance of hairlessness and sterility in mice exposed to ^{99m}Tc in milk secreted by lactating mothers.
- 5) Radiation effects observed in mice exposed in utero to radiation absorbed doses equivalent to those that might be received by the human fetus from diagnostic examinations of the mother with $\text{Na}^{99m}\text{TcO}_4$.
- 6) The toxicity studies must be extended to observations of any possible effects related to chemical toxicity of technetium (^{99}Tc) or to effects from radiocontaminants (equal to about 50 % of the β^- activity).

ACKNOWLEDGMENT

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DISCUSSION

Mary E. GAULDEN: Do you have any data on litter size of the various groups of progeny?

K.A. LATHROP: Yes, such data are available. No effect was seen in the F₁ groups, and my impression is that the attrition seen in other groups is due to infertility rather than to reduction in litter size.

R.O. McCLELLAN (Co-chairman): How would you place your work in relation to the quantity of radioactivity and the resulting radiation dose typically used in clinical applications in man?

K.A. LATHROP: About 20 mCi of ^{99m}Tc are used to perform a single diagnostic brain scan. The resulting radiation dose to the foetus, according to our best estimate, is approximately 2 rads.

H.S. DUCOFF: If the question of separation of radiation from chemical toxicity is important, could we not approach it by using poikilotherones, maintaining one group at low temperature for one or more intervals in order to minimize chemical effects without altering radiation dose?

K.A. LATHROP: This is a novel idea, which I should like to explore with you.

CANCERS DU POU MON PROVOQUES CHEZ LE RAT PAR LE RADON ET SES DESCENDANTS A DIVERSES CONCENTRATIONS

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Abstract-Résumé

LUNG CANCER INDUCED IN RATS BY RADON AND ITS DAUGHTER NUCLIDES AT DIFFERENT CONCENTRATIONS.

In previous experiments on rats, nearly all the animals inhaling radon and its daughter nuclides in high concentrations developed bronchopulmonary cancers. In the experiments described here, the dose-effect relationship was studied as a function of several parameters: influence of total inhaled doses which were lower than in the previous experiments; influence of the concentration of the gas and its state of equilibrium with its daughter nuclides; influence of the duration of daily and total exposure. There was a definite increase in the frequency of cancers as a function of cumulative exposure. The respective effects of active deposition and daily dose are shown quite clearly. In view of the considerable importance of the dose-effect relationship in the radiation protection of uranium miners, it seemed worth while to compare these different experimental findings with the results of epidemiological studies made in this category of workers.

CANCERS DU POU MON PROVOQUES CHEZ LE RAT PAR LE RADON ET SES DESCENDANTS A DIVERSES CONCENTRATIONS.

Lors d'expériences précédentes portant sur des rats, presque tous les animaux exposés à des inhalations de radon et de ses descendants à des concentrations élevées avaient présenté des cancers broncho-pulmonaires. Dans les expériences décrites, la relation dose-effet est étudiée en fonction de plusieurs paramètres: influence de doses totales inhalées plus faibles que dans les expériences précédentes; influence de la concentration du gaz, de son état d'équilibre avec ses descendants; influence de la durée d'exposition journalière ou globale. L'augmentation de fréquence des cancers en fonction de l'exposition cumulée est nette. Les influences du dépôt actif et de la dose quotidienne apparaissent nettement. Etant donné l'importance considérable que revêt la relation dose-effet dans la radioprotection des mineurs d'uranium, il a paru intéressant de rapprocher ces différents résultats expérimentaux de ceux qui ressortent des enquêtes épidémiologiques réalisées sur cette catégorie de travailleurs.

INTRODUCTION

Lors d'expériences précédentes portant sur des rats Sprague Dawley, nous avons provoqué l'apparition de cancers broncho-pulmonaires chez un grand nombre d'animaux. Ils avaient inhalé du radon à 30% de l'équilibre avec ses produits de filiation. L'exposition cumulée totale avait été de 9000 WLM¹ environ [1]. Les résultats que nous avons obtenus dans de

¹ Le WLM correspond à 170 heures de travail dans une atmosphère où l'on trouve une énergie potentielle de $1,3 \cdot 10^5$ MeV, quel que soit l'état d'équilibre du radon avec ses descendants.

nouvelles expériences avec des doses totales inhalées plus faibles nous autorisent à penser que ce type d'expérimentation est intéressant à un double point de vue. D'abord parce qu'il permet l'étude de la relation dose-effet dans les meilleures conditions, car, comme nous allons le voir, la méthode est simple et l'interprétation de la réponse à l'agression d'une grande sûreté, et aussi parce que ces données expérimentales pourraient présenter un grand intérêt dans la radioprotection des mineurs d'uranium si on pouvait les extrapoler à l'homme. C'est pourquoi nous comparerons la fréquence des cancers broncho-pulmonaires obtenus chez l'animal en fonction de la dose totale de radon inhalé à la fréquence des cancers du poumon des mineurs telle qu'elle ressort des enquêtes épidémiologiques [2 - 4].

MATERIEL ET METHODES

Dans une première période et jusqu'en 1972 nous avons utilisé un système d'inhalation très simple. La source de radon était produite par du minerai d'uranium riche finement broyé et étalé sur des claies superposées dans un caisson étanche. La chambre d'inhalation était constituée par une ou deux boîtes étanches en plexiglas. La chambre et la source étant reliées par deux canalisations, une pompe à membrane, installée sur l'une d'elles, assurait la circulation de l'air dans un circuit fermé. Dans ces conditions la concentration en radon obtenue était d'environ $7,5 \cdot 10^{-7}$ Ci/l pour un équilibre du radon avec ses descendants d'environ 30%, état d'équilibre pouvant descendre jusqu'à 1% si on plaçait un filtre sur le circuit. Ce système, qui avait l'avantage d'une très grande simplicité, ne permettait pas d'obtenir des expositions à des concentrations très élevées.

C'est pourquoi nous utilisons depuis trois ans de nouvelles installations. La source de radon est constituée par 57 fûts de sulfate de plomb radifère (2 Ci de radium par fût) entreposés dans deux grandes cuves en inox qui sont enterrées. Cette source est reliée par un système de canalisation à un caisson intermédiaire puis à deux chambres d'inhalation métalliques de 10 m^3 chacune, avec portes étanches. Chaque salle peut accueillir 300 rats. L'expérience est programmée grâce à un système électronique. Les séances d'inhalation peuvent durer jusqu'à 16 heures. Pour un fonctionnement journalier, on peut obtenir des concentrations allant de $3 \cdot 10^{-7}$ à $1 \cdot 10^{-6}$ Ci/l de radon à l'équilibre avec ses descendants. Ce nouveau système permet la mise en expérience d'un grand nombre d'animaux, ce qui revêt une grande importance pour apprécier l'effet de doses faibles et aussi de concentrations de gaz élevées et à l'équilibre [5].

On voit que dans l'un ou l'autre cas la méthode reste simple et ne présente pas de risque particulier pour l'opérateur si l'étanchéité du système est régulièrement contrôlée. Elle est aussi très souple puisqu'il est possible de faire varier très facilement la dose du cancérigène soit en diminuant la concentration du gaz, soit en modifiant son équilibre ainsi que son débit ou simplement en faisant varier la durée des séances d'inhalation.

Les animaux utilisés sont des rats Sprague Dawley SPF; ils sont âgés de trois mois au début des expériences. Le rat a été choisi car c'est un animal de laboratoire résistant, peu encombrant, peu coûteux et aussi, ce qui est très important, parce qu'il ne fait pratiquement jamais de cancer du poumon spontanément. Aucune tumeur maligne n'a été trouvée dans les

animaux témoins. Pendant la durée des expériences les animaux, qui sont pesés régulièrement, gardent un bon état général. Les poumons et les autres organes sont prélevés à la mort de l'animal ou après sacrifice si le rat présente des signes de mort imminente souvent précédés d'un amaigrissement brutal. La cause de la mort est souvent difficile à préciser, elle n'est pas infectieuse et, en cas de cancer du poumon, rarement provoquée par des complications tumorales. La durée de vie est d'autant plus écourtée que la dose totale de radon inhalée a été forte et le débit de dose élevé. La technique histologique du traitement des poumons est classique et permet l'examen du poumon entier [5].

INVENTAIRE HISTOLOGIQUE

Les lésions rencontrées au niveau des poumons sont de deux types:

a) Bénignes: métaplasie, adénomatoses et adénomes d'apparition précoce; pneumonie interstitielle surtout, dominant dans les huit premiers mois.

Malgré l'homogénéité de l'agression la pneumonie interstitielle évolue en foyers plus ou moins étendus à proximité de tissus apparemment peu modifiés au microscope optique. Du point de vue histologique les septa apparaissent très épaissis. Les fibres de réticuline et les fibres élastiques sont denses et fragmentées. Le revêtement alvéolaire est hyperplasique par prolifération du pneumocyte II. Des membranes hyalines éosinophiles en assises concentriques se forment. En dehors de ces foyers, le parenchyme paraît normal à l'exception de pneumocytes II hypertrophiés et de foyers de rétention macrophagique [6].

b) Malignes: elles apparaissent entre le 13^e et le 24^e mois après le début de l'inhalation [7]. Elles peuvent être de trois types, en distinguant les lignées cellulaires concernées: carcinome bronchogénique, carcinome bronchiolo-alvéolaire, très rarement sarcome.

RESULTATS EXPERIMENTAUX

Dans les expériences que nous allons présenter, qui avaient des buts différents, nous ne retiendrons que la fréquence d'apparition des différents types de tumeurs malignes en fonction de la dose inhalée. L'exposition totale cumulée est exprimée en WLM. Les premières expériences ont été effectuées avec la première installation, la troisième avec l'installation actuelle.

Dans l'expérience présentée dans la partie A du tableau I les animaux étaient exposés à du radon continuellement filtré pour éliminer l'action des produits de filiation. L'exposition cumulée se situe entre 300 et 500 WLM, compte tenu de la composante radon et de la difficulté de mesure du dépôt actif pour des équilibres aussi faibles. Cette expérience montre la responsabilité du dépôt actif.

La partie B du même tableau montre l'augmentation de fréquence des cancers pulmonaires avec l'augmentation de l'exposition cumulée. La moitié des animaux à plus haut niveau (9600 WLM) avaient inhalé en plus

TABLEAU I. NOMBRE DE CANCERS DU POUMON CHEZ LE RAT APRES INHALATION DE RADON

WLM	Nombre de rats	Lignée cellulaire			Cancers totaux	
		Broncho- génique	Bronchiolo- alvéolaire	Mixte	Nombre	%
<u>A. Concentration: 150 WLM -- Durée des séances: 5 heures</u>						
300 - 500	26	1	1		2	8
<u>B. Concentration: 2500 WLM -- Durée des séances: 5 heures</u>						
750	20	1	3		4	20
1500	20	3	2		5	25
3000	40	3	14		17	52
4500	40	12	14		26	65
9600	40	13	14	3	30	75
<u>C. Concentration: 3000 WLM -- Durée des séances: 16 heures</u>						
2000	25	4	3		7	28
3500	25	6	2	1	9	36
5500	25	4	3		7	28
7000	25	8			8	32

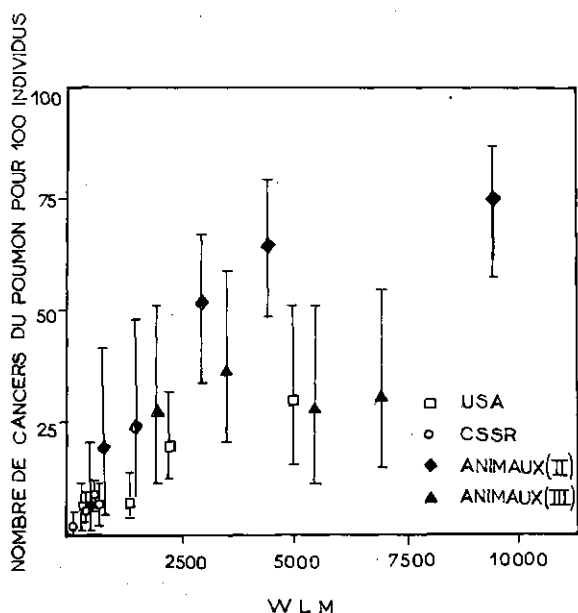


FIG. 1. Relation entre la dose et le nombre de cancers du poumon: résultats expérimentaux (II: rats à 2500 WLM, tableau I.B; III: rats à 3000 WLM, tableau I.C).

de la poussière de minerai d'uranium riche; le nombre de cancers observés étant égal à celui de la moitié n'ayant pas inhalé de poussière, les deux catégories ont été regroupées. L'expérience présentée dans la partie C du tableau montre l'influence des séances de longue durée. La comparaison des parties B et C met l'accent sur l'influence de la dose quotidienne.

Il est possible de comparer sur un même graphique les résultats exprimés dans le tableau I à ceux déjà publiés [3, 4] des enquêtes épidémiologiques (fig.1).

DISCUSSION

A la précision des différentes données près, les valeurs expérimentales et les données des enquêtes épidémiologiques s'intercalent parfaitement. Comme pour tous les éléments radioactifs, au-delà d'une certaine dose cumulée totale on observe avec le radon une diminution de la fréquence des cancers, qui apparaît à un niveau d'autant plus faible que la dose journalière est plus élevée. Ce phénomène est lié à un raccourcissement plus important de la durée de vie qui, lorsqu'on augmente la dose quotidienne, intervient également pour tous les niveaux de dose totale cumulée que nous avons explorés. Si l'on compare enfin la proportion relative des carcinomes bronchogéniques et à pneumocytes II, on observe, comme le montre le tableau, une prédominance de carcinomes bronchogéniques pour les doses totales les plus élevées.

CONCLUSION

Il ressort de ces données, en tenant compte de la durée de vie des espèces et dans certaines conditions expérimentales précises, que le rat est un excellent modèle biologique pour l'étude de la relation dose-effet. Nous menons actuellement des expériences dans lesquelles de grands nombres d'animaux présentent des expositions cumulées comparables à celles des mineurs d'uranium. Nous cherchons à savoir si à de tels niveaux des tumeurs malignes peuvent apparaître, mais aussi des lésions telles que pneumonies interstitielles, dont les conséquences en pathologie humaine sont très importantes.

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DISCUSSION

H.H. VOGEL, Jr.: Did your studies of lung-induced cancers in male Sprague-Dawley rats contain any mammary gland neoplasms in either the irradiated or the control groups? If so, what percentage of the rats showed such mammary gland tumours?

J.C. NENOT: Like all investigators using the rat as the experimental animal, we observed benign tumours of the mammary gland, the frequency of whose appearance was related to the animal's age. The rate of mammary cancers was lower than 1%. However, we never found an increase in this cancer in animals which had inhaled radon.

R.O. McCLELLAN (Co-chairman): It has been suggested that cigarette smoking plays an important role in the induction of lung cancer in uranium miners. In your paper you demonstrated a correlation between the incidence of lung cancer in rats, presumably not exposed to cigarette smoke, and man as a function of WLM of radon and radon daughter exposure. Do you have experimental data on the effects of cigarette smoke and radionuclide exposure in rats?

J.C. NENOT: We did in fact subject the rats to continued inhalation of α -emitting radionuclides and cigarette smoke, trying to deliver a "cigarette dose" equivalent to the consumption of one packet a day by man. The most informative experiment was carried out with ^{241}Am . The fact of the greatest importance is not so much the significant increase in lung cancers as the increase in all other cancers, especially osteosarcomas, and the shortening of the latent period for appearance of all cancers, pulmonary or extra-pulmonary.

EVALUATION OF RADIATION-INDUCED PULMONARY LESIONS BY SPIT AUTORADIOGRAPHY

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Abstract

EVALUATION OF RADIATION-INDUCED PULMONARY LESIONS BY SPIT AUTORADIOGRAPHY.

Internal irradiation caused by radionuclides incorporated in pulmonary tissue is guilty of producing two kinds of biological effect: pulmonary fibrosis and carcinogenesis. The role of irradiation in the etheopathogenesis of carcinogenesis should be argued first by proving the presence of contamination radionuclides in pulmon, which is revealed by spit eliminations. To prove the presence of contaminating radionuclides inhaled in pulmon, spit autoradiography was used, representing the only way of showing the presence in pulmon of α - and β -emitting elements. Active elements contained in spit samples were identified by drawing the histograms of the path length of particles. Contamination was considered significant when the difference between average density of α traces determined on sample and average density of traces on control exceeded two to three times the average fluctuation. In the case of contamination with β -emitting elements, specific autoradiographic images were noticed for accumulations of β activity at cellular level. Spit autoradiography was applied during complex periodic medical examinations of miners working in radioactive ore mines. By means of exhaustive clinical and laboratory examinations, correlations were established between autoradiographic findings and pulmonary radiopathological aspects. At present, methodological improvements are being dealt with to standardize spit smear collecting and processing methods and especially to obtain exact quantitative determinations of pulmonary contamination. In this way it is hoped to establish a dose-effect relation that would be an objective element in considering pulmonary lesions caused by internal irradiation.

Internal irradiation caused by radionuclides incorporated in pulmonary tissue is guilty of producing two kinds of biological effect: pulmonary fibrosis and carcinogenesis. Pulmonary fibrosis caused by irradiation implies a high dose absorption; this seldom happens in cases of pulmonary contamination.

The relation has not yet been established between absorbed low-level doses, as a result of pulmonary incorporation of some radionuclides, and carcinogenesis. This effect is quantal in character and depends on probability per unit dose and total dose over the whole range from zero dose upwards. Thus, in attempting a full assessment of tumour induction by low-level doses, the main problem is to estimate a dose-response relation within an acceptable limit.

To evaluate the doses to which the lungs will be subjected as a result of incorporating various amounts of radionuclides, the presence of

contaminating radionuclides in pulmon must first be proved. To prove this we have used spit autoradiography, which is, up to now, the only way to show the presence of contaminating radionuclides in pulmon for α - and β -emitting radionuclides inhaled in the form of insoluble compounds which exhibit low transportability.

Spit was collected in Petri boxes for 24 hours for each person. The spit obtained was centrifuged for 10 minutes at 2000 rpm. Out of the sediment of cells or cellular remains, smears were effected on normal plates. The smears were deposited on a central rectangle of 1 cm² surface. The plates were processed by the standard method.

By an optic microscope with an immersion objective, on dry plates, a number of tracks were detected on a 70-100 mm² surface representing 12 displacements of the microscopic field on the central surface where the spit was deposited. Alpha-active elements contained in the spit samples were identified by drawing histograms of the track lengths of α particles. The track lengths in the Ilford or IFA.EN.3 emulsion was placed between 14 and 35 μ m for the majority of α -active natural elements. The histogram of the track length should present maxima for uranium and its natural descendants, as shown in Table I.

Figure 1 shows a histogram for identifying radionuclides depending on the position of maxima. The α tracks are easily recognized on the autoradiograph by their rectilinear aspect.

Radioactive contamination of the spit was estimated for track density per surface unit of the measured and control probes (containing spit from healthy people).

The control average for each measured series and standard deviation (α -track fluctuations were calculated to see whether or not the measured track numbers corresponded to real spit contamination). The difference was calculated between the track density determined on each sample and the control average.

The difference between the track density of the measured and the control probe corresponds to a real contamination when this difference exceeds three times the track fluctuation determined on control plates. Where there were more plates of the same spit sample (2-3) the average of the track density was taken into account to see the degree of significance of the result.

TABLE I. TRACK LENGTHS OF VARIOUS RADIONUCLIDES

Radionuclide	Energy (MeV)	Track length in emulsion (μ m)
²³⁸ U	4.18	15.5
²³⁴ U	4.76	18.7
²²⁶ Ra	4.77	19.2
²²² Rn	5.49	23.5
²¹⁸ RaA	6.00	26.7
²¹⁴ RaC'	7.68	39.4
²¹⁰ Po	5.30	22.6

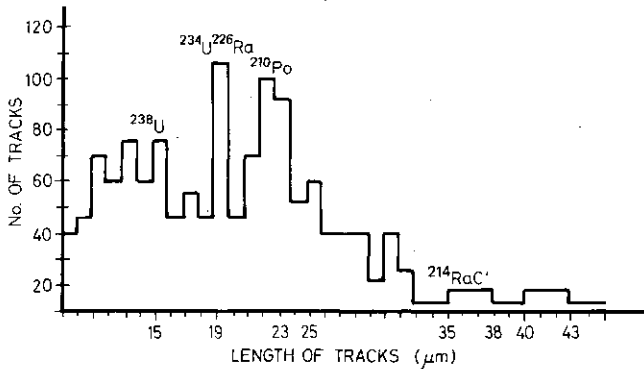


FIG. 1. Histogram for identifying radionuclides depending on the position of maxima.

A radioactive contamination therefore exists when the following condition is achieved:

$$D_P - D_M \geq 3 \sqrt{T_P^2 + T_M^2} = 3T$$

where

D_P = average track density on measured plates for a given probe

D_M = average track density on control probe

T_P = fluctuation of track density on probe

T_M = fluctuation of track density on control

Contamination was considered non-significant when the difference $D_P - D_M < 3T$ and significant when this difference exceeded three times the control fluctuation. Five or six control probes were performed for each series of autoradiographs. Fluctuation was also calculated.

To establish the sensitivity of the method, spit samples were prepared to which quantities determined from uranium saline solution were added. A solution of 0.4% uranyl acetate was prepared, of which a drop of 0.05 mlitre was introduced in 2 mlitres of spit from uncontaminated persons. In this way, each mlitre of contaminated spit contained 6.1×10^{-5} g uranium. Estimating to 0.01 mlitre the quantity of spit deposited on a plate in the central rectangle (100 smears can be made out of 1 mlitre spit), the result was that every standard plate contained 6.1×10^{-7} g U/cm².

The standard plates contaminated with uranium were covered with a sheet of 50 μm nuclear emulsion and treated similarly to the other probes.

Table II gives an example of the processing results for the α-track density on standard probes.

Relatively big differences were noticed from one probe to another, although they contained the same quantity of uranium. This variation was due to the very non-uniform uranium distribution on the smear, this being proved by the fluctuation of the number of α tracks in probes from one microscopic field to another.

TABLE II. NUMBER OF ALPHA TRACKS ON STANDARD PROBES

Probe No.	Conc. g U/mlitre spit	Conc. g U/cm ² plate	Surface (cm ²)	D _M average track density on control	D _p standard track density on probe	Difference D _p -D _M
E. 1	6.1×10^{-5}	6.1×10^{-7}	0.760	138.27 ± 20.05	1569	1431
E. 2	6.1×10^{-5}	6.1×10^{-7}	0.727	138.27 ± 20.05	2153	2015
E. 3	6.1×10^{-5}	6.1×10^{-7}	0.727	138.27 ± 20.05	2137	1999
E. 4	6.1×10^{-5}	6.1×10^{-7}	0.704	138.27 ± 20.05	2260	2122
E. 5	6.1×10^{-5}	6.1×10^{-7}	0.780	138.27 ± 20.05	1619	1491
E average					2149	2011

For this reason, we used in one calculation the fluctuation of the number of α tracks, considering 2000 tracks/cm² for each standard plate.

If we considered as significant the difference D of the α track density on the probes as compared with the control probes for about 60 tracks (3 standard values of the fluctuation of the number of α tracks on control) we calculated the minimum concentration of uranium detectable by autoradiography:

$$2000 \alpha \text{ tracks/cm}^2 \text{ represent } 6.1 \times 10^{-7} \text{ g U/cm}^2$$

$$3 \times 20 \text{ tracks/cm}^2 \text{ represent } x$$

and

$$x = \text{the sensitivity of the method} = 18.3 \times 10^{-9} \text{ g U/cm}^2$$

For this quantity of uranium the following activity may be estimated when utilizing the relation:

$$\Lambda = \lambda N \frac{1}{3.7 \times 10^{10}}$$

where

Λ = activity (Ci)

λ = decay constant

N = nucleus number/g

resulting in:

$$\Lambda(\text{gU}) = 33.6954 \times 10^{-3} \text{ Ci}$$

and for $18.3 \times 10^{-9} \text{ g}$ there results the activity of $6.17 \times 10^{-3} \text{ pCi}$ which may be considered as the minimum threshold of the detecting sensitivity for autoradiography.

For comparison, we determined the sensitivity of a measuring method with scintillation detector SZn(Ag), resulting in a minimum detecting threshold of 334×10^{-3} pCi. This proves that the autoradiographic method is about 55 times more sensitive. In such conditions autoradiography can be considered as a selection method in estimating the biological effects of low-level doses. Moreover, on some plates autoradiographic images could be seen, characteristic of accumulation at cellular level of a β emission. On the plates from uranium miners, these images were interpreted as a result of an agglomeration of RaD (^{214}Pb) from radon descendants.

We have applied spit autoradiography, among other methods, in the periodic medical examination of uranium miners. The cases presenting significant contamination were selected by spit autoradiography.

Exhaustive clinical and biological investigations were performed to detect possible cases of pulmonary neoplasm. All the clinical and radiological investigations proved insufficient to make a full assessment of the pulmonary neoplasm diagnosis in the case of silicotic lung. In an attempt to overcome this difficulty, we are trying to evaluate those data provided by spit autoradiography which help to predict the diagnosis of tumour induction.

It should be emphasized that radioactive contamination was demonstrated by spit autoradiography from a qualitative point of view without a quantitative estimate of the radioactive pulmonary load. On the basis of some experimental data and clinical experience, we are attempting to set the rate parameters of elimination by spit for some radionuclides to try to deduce the pulmonary load. This may be the only possible way to calculate the dose rate at the level of pulmonary tissue.

Because there is as yet no very exact evidence that tumour induction is linearly related to tissue dose in man, we are obliged to accept the existence of a threshold dose for tumour induction. But, so long as dose, dose rate and LET are not exactly estimated at the level of deposition of α particles in pulmonary tissue, there seems some pragmatic justification for using spit autoradiography as a basis for establishing a threshold for the potential hazard of pulmonary carcinogenesis in man. As we know, confirmation of an advanced pulmonary tumour is only of documentary interest. By using spit autoradiography in early detection methodology of lung cancer, we are, however, attempting to point out its utility in estimating this threshold of potential hazard. Thus, for everybody whose spit autoradiography showed significant contamination, we also made spit cytological investigations.

For example: S.V., 40 years old, has been working in a radioactive environment for ten years; normal radiological and clinical investigations. Spit autoradiography showed significant contamination (exceeding eight times the control fluctuation). The spit cytological investigation showed up cells of tumoral aspect. Bronchoscopy: Left bronchial tree is diffusely congested, without parietal infiltration in explorable areas. Upper lobar bronchus with low mobility from where cytological material was scraped. Normal right bronchoscopic aspect. Subsequent developments confirmed the diagnosis of pulmonary neoplasm.

On the basis of a large amount of early detected cases, by means of autoradiographic and cytological investigations of the spit, we are trying to establish the quantitative relation between the degree of contamination of the spit (expressed as the amount of standard errors) and the probability of tumour induction. However, we consider it logical to subject to an exhaustive periodic medical examination all those whose spit autoradiography shows contamination exceeding three times the control fluctuation.

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DISCUSSION

R.O. McCLELLAN (Co-chairman): Have you carried out serial observations on the same individual?

C. PAUN: I have not done so yet, but I intend to perform autoradiography on all the miners during their periodic medical examinations.

EXTRAPOLATION OF ANIMAL RADIONUCLIDE RETENTION DATA TO MAN

Use of similarity ratios*

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Abstract

EXTRAPOLATION OF ANIMAL RADIONUCLIDE RETENTION DATA TO MAN: USE OF SIMILARITY RATIOS.

A major problem in toxicology is the need for testing a myriad possible substances on several animal species as an aid in establishing standards for man. Particular interest in the problem of extrapolation to man is linked to a need for a method of obtaining transfer coefficients in food-chain models of ecosystems. Such a method could circumvent the necessity to test many species and compounds. Because of their particular interest in radioecology, the authors' studies were initiated using radionuclide retention data available in the literature. Since radionuclide retention should be a function of metabolism it follows that direct or indirect measures could be described by a "power law" based on body weight of the organism(s). However, when such power laws have been extended to interspecies comparisons the resulting power coefficients are usually substantially less than the value anticipated and seem to be different for each radionuclide. This paper proposes that interspecies comparisons ought to be based on the proportionality coefficient rather than the power parameter of the power function model and have called pairwise comparisons amongst species "similarity ratios". Retention data were examined from five non-ruminant species (including man) where several radionuclides with different physical properties were fed. Subsequently an expression was devised whereby an estimate of biological equilibrium level in man could be calculated using similar estimates from experiments using mice, rats or dogs. There are some statistical questions to resolve which have to do with the assumed frequency distribution for estimates of the proportionality coefficient. In addition, repeated use was made of the same data sets.

INTRODUCTION

The basic ideas used in this paper were previously developed and reported [1]. The focus of that study was the extension of the concepts of biological similarity and allometry to deal with food-chain problems, with particular emphasis on radioactive substances in the environment. The methodology was later applied, using some additional radionuclides [2], and the results were discussed with reference to environmental pollution. In the present paper we focus on the applicability of the technique for the extrapolation of radionuclide retention data obtained from various laboratory animal species to one another and finally to man. While other workers [3,4] have considered body weight as a "normalizing device" or as a variable [5] for interspecies comparisons, we believe our procedure offers the advantage of a more direct connection to metabolism. The statistical limitations of our work are considerable (both in theory and because few data sets are available), but the need for such attempts is clear inasmuch as "extrapolation to man from animal data is admittedly difficult and sometimes inaccurate; however, the risks in not extrapolating are unquestionably greater" [6].

* Research done under contract with US Energy Research and Development Administration E(45-1).

TABLE I. Isotope characteristics, animal weights, and estimates of the coefficients in $y = \alpha w^{\beta}$ from other studies.

Isotope	Physical Half-time	Average Body Weight (kg) ^a /Species					Estimate of α and β		Reference
		Mouse	Rat	Dog	Monkey	Man	(a)	(b)	
²² Na	2.58 years	.0250	.2500	---	---	70 ^b	---	---	Richmond (1958)[10]
⁸⁶ Rb	18.7 days	.0250	.2500	---	---	70 ^b	---	---	Richmond (1958)[10]
¹³⁷ Cs	30 years	.0203	.3105	11.8	4.6	71.1	0.80	0.46	Richmond, et al. (1961) ^f
⁶⁵ Zn	245 days	.0162	.2775	10.9	---	67.9	1.55	0.38	Richmond, et al. (1962) ^f
¹³³ Ba	7.5 years	.0212	.3100	13.6	---	---	---	---	Richmond, et al. (1962)[11]
¹³¹ I	8.1 days	.0210	.2800	11.5	10.3	70 ^c	0.36	0.37	Furber and Richmond (1963) ^f
⁴⁰ K	over 10 years	.0370	.3150	---	---	57.8	0.45	0.45	Fujita, et al. (1966) ^f
⁵⁴ Mn	310 days	.0245	.3370	6.9	16.5 ^d	---	0.22	0.23	Furchner, et al. (1966) ^f
^{110m} Ag	249 days	.0265	.3350	13.3	6.8	70 ^c	0.10	0.28	Furchner, et al. (1968) ^f
¹⁰⁶ Ru	1 year	.0220	.2640	10.6	7.7	---	1.15	0.15 ^e	Furchner, et al. (1971)[12]

^aSome means represent group averages from more than one experiment while others are averages of individual replicate observations within an experiment.

^bEstimated from a surface area--body weight--height nomogram.

^cEstimated.

^dMature weight.

^eNot significantly different from zero.

^fFor citations see Thomas and Eberhardt [2].

METHODS

Our main emphasis is on the application of the "power-law"

$$y = \alpha w^{\beta} \quad (1)$$

to the analysis of data on radionuclide uptake and retention. The applicability of Equation (1) to organ growth and to metabolic functions has been demonstrated [7,8]. Recent work[9] has added an apparent theoretical foundation for using a value of 0.75 as an estimate of β in Equation (1) above, substantiating the empirical observations of others [8]. Since many laboratory studies are carried out with animals of nearly uniform size, body weights have mostly been considered only in terms of interspecies comparisons (cf. [5]). However, in such comparisons between species, the estimates of β have been considerably smaller than 0.75 (Table I). We have previously reported[1] that this result is not surprising because of the wide range of interspecies relative to intraspecies body weights of animals used for such studies (cf. Table I).

We have used both biological equilibrium level and long component biological half-time (calculated from the references in Table I) as dependent variables in Equation (1) above. Thus, for each animal for which multicompartment exponential fit to whole-body counting data was available, we were able to calculate two

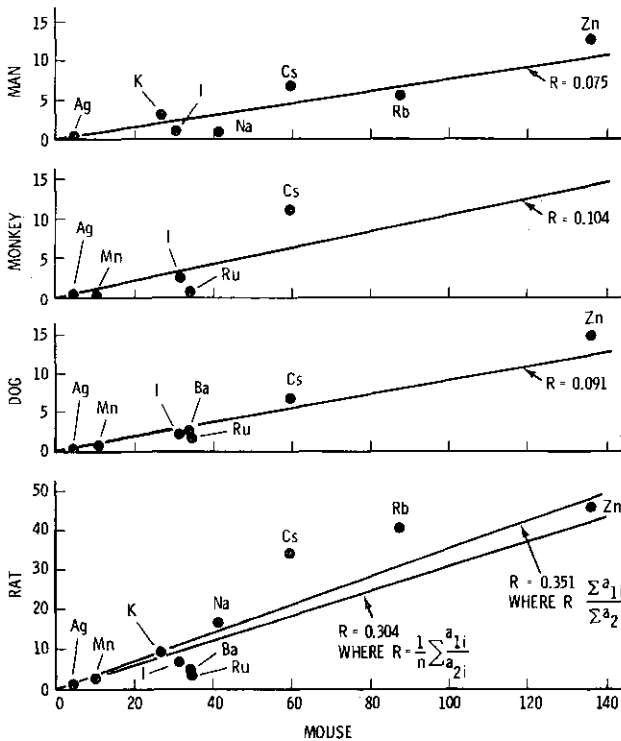


FIG. 1. Comparisons of a in biological equilibrium level = $\alpha w^{0.75}$ (similarity ratios) for mouse versus rat, dog, monkey and man.

TABLE II. Estimates of α (a) in $y = \alpha w^{0.75}$ where y = biological equilibrium level or long-component biological half-time.

Isotope	Biological Equilibrium Level					Long-Component Half-time				
	Mouse	Rat	Dog	Monkey	Man	Mouse	Rat	Dog	Monkey	Man
^{22}Na	42	17	---	---	0.7	717	297	---	---	19
^{86}Rb	87	40	---	---	5	64	11	---	---	4
^{137}Cs	60	34	6	11	7	112	34	6	9	5
^{65}Zn	136	46	15	---	12	3180	288	26	---	18
$^{133}\text{Ba}^a$	34	5	2	---	---	11924 ^c	1485	226	---	---
^{131}I	32	6	2	2	1	248	56	3	11	4
^{40}K	27	9	---	---	3	---	---	---	---	---
^{54}Mn	11	3	0.6	0.2	---	3118	626	21	18	---
^{110}Ag	5	1	0.4	0.2	0.2	1591	221	6	17	2
^{106}Ru	35	3	2	0.4	---	14290	1958	310	45	---

^aLast component was not used because of apparent deposition in bone.

^bUnavailable from Fujita et al. (1966).

^cUsed long-component half-time.

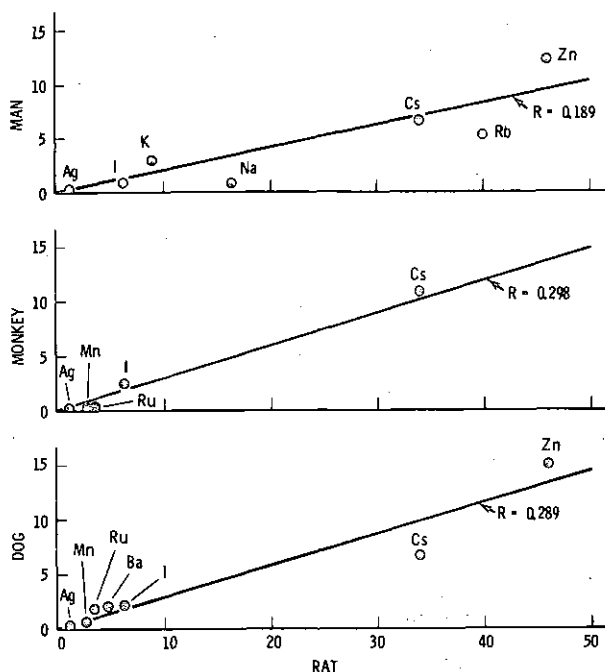


FIG. 2. Comparison of a in biological equilibrium level = $a_w^{0.75}$ (similarity ratios) for rat versus dog, monkey and man.

estimates of α (for sample values we use the letter a). We chose to use only data where oral isotope administration was used with nonruminant mammalian species and a long period of observation followed. The term "similarity ratio" [1] is used to refer to the slope of a line fitted to sets of estimates of α [Equation (1)] obtained for two species and a number of different radionuclides (see Figure 1). The statistical problems in fitting the ratio are discussed below.

RESULTS

The calculated values of a for each dependent variable are in Table II and the resulting similarity ratios are in Figures 1 and 2 for biological equilibrium level and Figures 3 and 4 for long-component biological half-time. The similarity ratio estimates (R) were calculated as:

$$R = \frac{\sum a_{1i}}{\sum a_{2i}} \quad (2)$$

where the subscripts 1 and 2 refer to the two species, and i denotes the i^{th} radionuclide. For comparison an alternate estimator:

$$R = \frac{1}{n} \sum \frac{a_{1i}}{a_{2i}} \quad (3)$$

was used in portions of Figures 1 and 3. Since both the numerators and denominators in Equation (2) or (3) are from unknown statistical

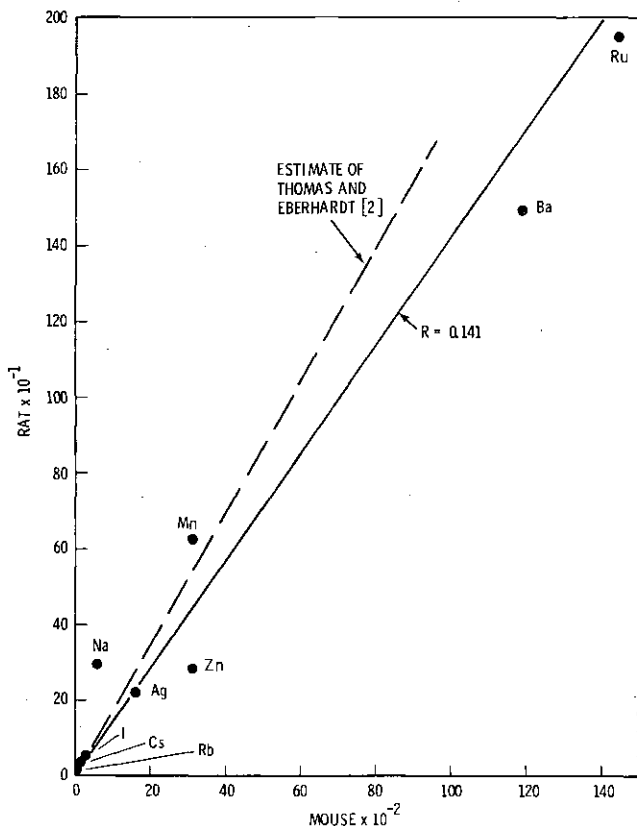


FIG. 3. Comparison of a in long-component biological half-time (similarity ratio) for mouse versus rat.

distributions, the choice of estimator for R requires further study (there are some circumstances[13] in which the above estimators would be appropriate).

Although each of the radionuclides shown in the figures serve different physiological functions there seems to be a definite relationship for either mouse (Figure 1) or rat (Figure 2) when compared to larger species. The upper three panels in Figure 1 indicate that the value of the similarity ratios are nearly the same while the comparison between mouse and rat exhibits a larger value of R . Because of the relative consistency of the upper three comparisons in Figure 1, it is not surprising that similarity ratios for all larger species compared with rats (Figure 2) are reasonably alike. In fact, the comparisons in Figure 2 could be calculated directly from the ratios depicted in Figure 1 if there were equal sample sizes for each species comparison. The reason for the observed consistency (Figure 1) seems to be due to the failure of the numerator in Equations (2) or (3) to be very different "within an isotope" for the larger three species (Figure 5). In general, the value of a declines with increasing species weight, but the differences between dog, monkey and man are

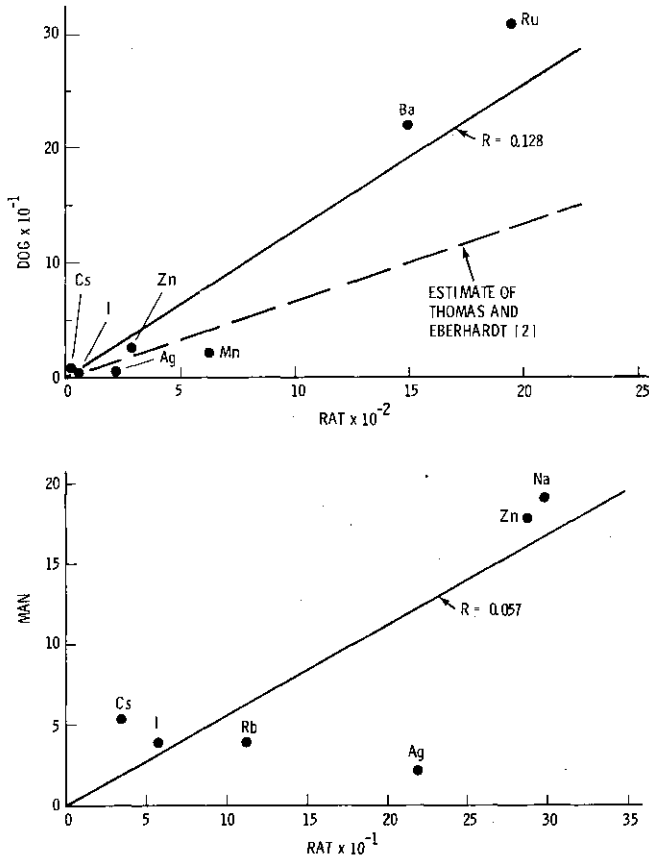


FIG. 4. Comparison of a in long-component biological half-time (similarity ratios) for rat versus man and dog.

not numerically large. Thus, we have combined the information in Figure 1 for the larger species into an overall comparison with the mouse data (Figure 6). The data point for monkeys fed a single oral dose of ^{137}Cs seems to be brought into question in this case, as does perhaps the observation on sodium in man.

The similarity ratios for mouse vs. rat in Figure 3, using long-component biological half-time, are much more encouraging than presented earlier [2], where less data was available. However, the comparisons for rat vs. dog or man (Figure 4) seem to indicate that there is only a tenuous relationship at low a values and only two data points are available at higher values.

Additional data for man and dog will be necessary to be sure a relationship exists. The fact that biological equilibrium level seems to give more consistent similarity ratios is not surprising, since it is computed using the entire exponential retention function,

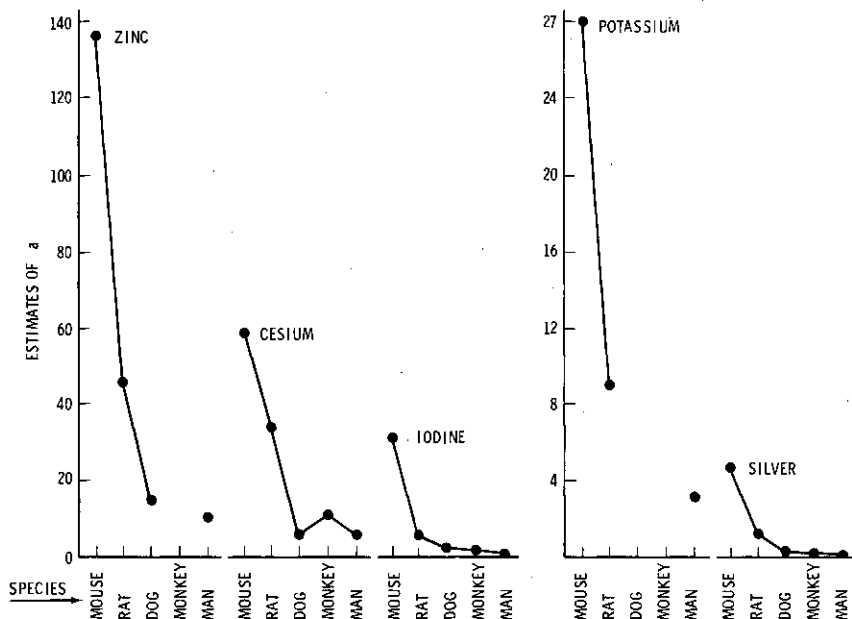


FIG. 5. Association of a in biological equilibrium level $= aw^{0.75}$ with species for several radionuclides.

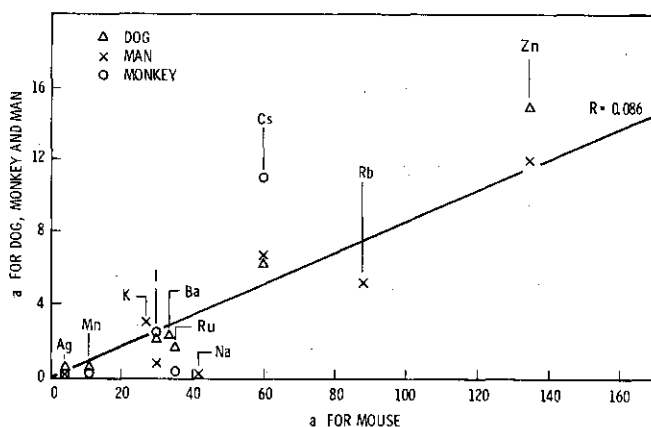


FIG. 6. Similarity ratio for mouse versus dog, monkey and man.

while long-component half-time describes only a portion of the metabolic turnover of radionuclides. Further, there is no consideration of pool size when half-time is used.

DISCUSSION

The general methods which are used in toxicology to aid in extrapolation to man usually consist of laboratory trials using several species (normally at several dose levels) followed by an

attempt to extrapolate results using body weight, surface area, metabolic rate, dose/kg or some other independent variable. This information, in conjunction with studies of metabolic fate, biochemical and/or physiological similarities, and basic mechanistic studies are used to set standards for, or predict results, in man. This procedure is formidably expensive and involves testing a myriad of compounds in many species. If our procedure, employing similarity ratios, could be used for other compounds, metabolic end points, and the statistical questions resolved, a great deal of the step-by-step testing could be circumvented. As an illustration, suppose a similarity relationship such as that depicted in upper panel of Figures 1 and 2 or the lower panel of Figure 4 is available. The only biological test necessary would entail using either mice or rats, followed by calculation of a in Equation (1), and reading from the appropriate graph to obtain a comparable value for man. Thus, additional testing in other species might either be circumvented, or at least delayed, while other confirmatory metabolic studies are conducted with mice to judge their adequacy as a biochemical or physiological stand-in for man. A rather small trial with dogs or monkeys might be conducted to confirm the foregoing results.

SUMMARY

We have shown that the ideas of similarity analysis appear to exhibit considerable promise as a device to extrapolate radionuclide retention data among various nonruminant mammalian species including man. Our analysis leads us to speculate that similarity ratios for other classes of compounds may also be useful if the statistical questions can be resolved.

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DISCUSSION

R.O. McCLELLAN (Co-chairman): I note that all your data were derived from omnivores or animals kept on mixed diets. It would be useful to see how the data fit if you include a herbivore, particularly when considering alkali metals.

J.M. THOMAS: If our technique is to be useful (at least to this point), we need data based on at least three (and hopefully more) radionuclides fed to two common species. Moreover, the rules we have used until now require a single oral administration of the isotope(s) followed by a long observation period to estimate retention. The ruminant species for which we have obtained usable data in accordance with the above rules have the same retention information available either for only one isotope and several species (i.e. ^{137}Cs in sheep, mice and rats) or other "unmatched" ruminants (i.e. ^{131}I in goats).

R.O. McCLELLAN: All the data you presented dealt with elements apparently administered in a simple form. Have you had the opportunity to consider more complex organic compounds?

J.M. THOMAS: No, but we would like to utilize some of the data given in Refs [3] and [4] in our paper in conjunction with our similarity values to modify, say, species LD-50 values. The final extension of interest would be to an interspecies comparison of tumour incidence.

M.N. KHALANSKI: What would be the influence of the age of the animals on the transfer of elements, especially iodine?

J.M. THOMAS: We have not specifically considered this question, but the range of body weight for dogs and particularly monkeys in such that age is a factor in the analysis. We have not evaluated the effect of age for iodine in particular, but believe it would not be a serious problem except for very young or very old animals.

J. SCHUBERT: What is the variation in the constants of your equation or the variability if you use different strains of the same species?

J.M. THOMAS: To the extent that the animals used in these studies were from experiments conducted in some cases at periods as much as ten years apart, and in different laboratories, it is reasonable to assume that the results reflect at least some strain differences. How this affects the variability in R is uncertain. We have discussed the statistical problems in simply estimating R in the paper, and suppose that real strain differences would make this an even more complex issue.

A.L. BROOKS: How would the constants be affected if you compared species which retain a certain isotope or elements with very different half-lives? For example, the dog and the Chinese hamster retain ^{144}Ce or ^{239}Pu in the liver with a long effective half-life, while rats and mice clear these elements very rapidly.

J.M. THOMAS: We have not studied either cerium or plutonium, but find that the same sort of differences in the retention time for the long component exist in the case of whole-body retention data among species for the elements studied. For some radionuclides, rats and mice had shorter long-component half-times (^{65}Zn , ^{137}Cs , ^{22}Na , ^{86}Rb) than larger animals, while for other elements (^{131}I , ^{106}Ru) the half-time seemed unrelated to species size. Lastly, there were two radionuclides (^{54}Mn and $^{110\text{m}}\text{Ag}$) where the long-component retention time was longer for mice and rats than for the larger animals.

P.G. GROER: Alkaline-earth metabolism has been studied extensively. Have you tried your methods for these nuclides? This would provide a rather interesting check.

J.M. THOMAS: No, we have not, except for ^{133}Ba . Even though there is an abundance of data available on strontium and radium in several species we were unable to find sets involving a single oral ingestion followed by whole-body retention estimates performed for a long time. Furthermore, reference to Table I in the paper indicates we had some difficulty with the ^{133}Ba data.

HUMAN STUDIES
(Session VIII)

Co-Chairmen:

M.L. GRIEM (United States of America)

A. LUZ (Federal Republic of Germany)

AN INVESTIGATION OF EFFECTS OF PRE-NATAL EXPOSURE TO DIAGNOSTIC X RAYS

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Abstract

AN INVESTIGATION OF EFFECTS OF PRENATAL EXPOSURE TO DIAGNOSTIC X RAYS.

A number of epidemiological studies have demonstrated a relationship between prenatal or pre-conceptual exposure to diagnostic X rays and the incidence of leukaemia or other forms of cancer. Radiation exposure in these studies was always prompted by a medical indication, and this may have introduced biases in the selection process to which the observed effects might be attributed. A prospective long-term follow-up study was carried out of about 1000 children exposed in utero to routine pelvimetry examinations at the Chicago Lying-In Hospital, along with suitable controls. This study should have been relatively free of bias since medical indication played no role in the selection process. Data on all significant illnesses and hospitalizations were collected and analysed, and no conclusive evidence of a radiation effect was found. These results were compared with those of Diamond and Lilienfeld, who found a doubling of the gross death rate among the white children in their study who were exposed to diagnostic X rays in utero. No such effect was found; furthermore, the discrepancy between the results of Diamond and Lilienfeld and of the present authors is statistically significant. Differences in the methods of selection appear to account for this discrepancy. The authors also compared other studies of effects of diagnostic X rays with studies in which exposure occurred on a non-selective basis from atomic bomb radiation, and found discrepant results in each instance. This suggests that the various reported effects of exposure to diagnostic X rays might actually be attributable to biases introduced by the selection process.

While the effects of radiation at high dose levels are well established, there is considerable disagreement about the magnitude of these effects at low dose levels, in the range of 1/2 rad to 5 rads. Experimental data are scanty here, and most of the evidence is based on epidemiological studies of human exposure to diagnostic x-rays, especially exposure occurring prior to birth and even prior to conception. These studies will be examined critically, with particular emphasis on possible biasing factors.

REPORTED EFFECTS

Nearly 20 years ago, Stewart et al. [1] reported the results of a retrospective study of 1299 children in the British Isles who had developed leukemia or other forms of cancer, and of an equal number of matched controls. Mothers of these children were contacted and were asked, among other items, about x-ray exposure to the abdomen during the pertinent pregnancy. Stewart found that 13.7% of the children with cancer had been x-rayed in utero, as compared with 7.2% of the controls. This corresponds to a relative risk of cancer, following such exposure, of 2.0.

* Operated by the University of Chicago for the US Energy Research and Development Administration.

TABLE I. CHILDHOOD LEUKEMIA FOLLOWING INTRAUTERINE EXPOSURE TO DIAGNOSTIC X-RAYS

Study	Leukemia Group			Control Group			Relative risk
	Total	X-rayed in utero	Rate (%)	Total	X-rayed in utero	Rate (%)	
Ford et al. [2]	78	21	26.9	306	56	18.3	1.64
Polhemus and Koch [3]	251	69	27.5	251	58	23.1	1.26
MacMahon [4]	304	47	15.5	7242	770	10.6	1.55
Graham et al. [5]	313	27	8.6	854	54	6.3	1.40
Stewart and Kneale [6]	2947	458	15.5	6347 ^a	645	10.2	1.61

^aMatched controls for all cancers.

This study stimulated a number of independent studies of the relationship between intrauterine irradiation and leukemia or other cancers. The largest of these [2-5] are listed in Table I. They verify the existence of an elevated relative risk of leukemia following intrauterine x-ray exposure, with values ranging from 1.3 to 1.6. Two of these studies [2,4] confirmed the increased risk for cancer other than leukemia. Stewart continued her study, and for a considerably expanded group of cancer and control cases found the relative risk to be 1.6 [6]. A few studies [7,8] failed to show an elevated risk of leukemia following intrauterine x-ray exposure; in each instance, however, the number of children with leukemia was small, and the results were not inconsistent with an increased risk.

Prenatal exposure to diagnostic x-rays has been associated with other effects. In a prospective study, Diamond and Lilienfeld [9,10] found that intrauterine exposure was associated with a doubling of the risk of death from all possible causes among exposed white children. Meyer et al. [11] followed up the black children in Diamond and Lilienfeld's study and found a significantly increased proportion of male offspring of women who themselves had received radiation in utero before 30 weeks of gestation. Graham et al. [5] observed that irradiation of the mother before the child had been conceived was associated with an increased risk of leukemia similar to the increase found following intrauterine exposure. Uchida and Curtis [12] reported that such pre-conception irradiation was associated with a fourfold increase in the risk of mongolism.

POTENTIAL SOURCES OF BIAS

The studies mentioned thus far are subject to criticism on the grounds that exposed and unexposed children differ with respect to factors other than their history of x-ray exposure. In particular, the x-rays were always obtained because of some medical indication; therefore one may anticipate that the exposed mothers collectively had a higher incidence of medical problems than the unexposed mothers. This is borne out by data of Diamond and Lilienfeld [9], given in Table II. In all instances, the proportion of births with complications during pregnancy or operative delivery was significantly higher for the exposed than for the control subjects. It would not be surprising if the exposed children as a group were less healthy than the control children solely on the basis of the factors that led to the x-rays.

TABLE II. COMPLICATIONS OF PREGNANCY AND OPERATIVE DELIVERY RELATED TO INTRAUTERINE EXPOSURE TO DIAGNOSTIC X-RAYS
Data of Diamond and Lilienfeld [9]

	Exposed		Control	
	No./total	Rate (%)	No./total	Rate (%)
Complications of pregnancy				
White subjects	2367/5262	45.0	2689/10337	26.0
Black subjects	5066/8911	56.9	5648/14978	37.7
Operative delivery				
White subjects	1476/5259	28.1	780/10338	7.5
Black subjects	1489/8912	16.7	530/14979	3.5

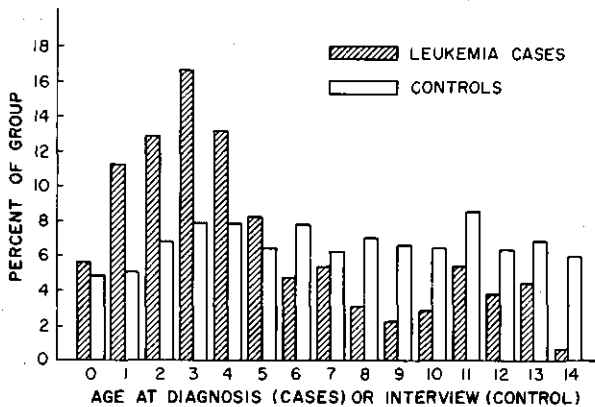


FIG. 1. Distribution of ages of leukaemia cases and controls (Graham et al. [5]).

More subtle biases can creep into a study and may not be found unless carefully looked for. Meyer and Tonascia [13] studied a group of 1458 black children who were exposed to x-rays in utero, along with an equal number of matched controls. On careful inspection of their data, they found evidence for a socioeconomic bias. Whereas 14% of the control mothers were admitted as private or semiprivate patients, only 2% of the exposed mothers had this hospital pay status (the remainder were admitted as ward or welfare patients). Since many diseases and conditions correlate with socioeconomic factors, an imbalance of these factors could bias the results.

An age bias was present in the study of Graham et al. [5], as shown in Fig. 1. The ages of the control children were fairly evenly distributed between 0 and 14 years, while the ages of the leukemic children were concentrated in early childhood, since this is the period when childhood leukemia tends to occur. The time interval from pregnancy to interview was, on the average, somewhat shorter for the leukemic children than for the controls.

One would therefore expect mothers of leukemic children to have less difficulty in recalling facts about their pregnancy, such as x-ray exposure, than mothers of controls.

Epidemiological studies are much more likely to contain biases than experimental studies. Often these biases can be discovered only by careful searching; sometimes it is impossible to identify them because the necessary data are unavailable. When biases are identified, their effects can be offset by stratification of the samples, as was done by Graham et al. [5], to compensate for unequal age distribution and various other factors. Despite such efforts, the possibility that observed effects may be due to unrecognized or inadequately handled biasing factors can never be completely excluded.

THE UNIVERSITY OF CHICAGO STUDY

We have carried out a study at The University of Chicago in which biases due to the selection process should be minimal [14]. This is a prospective long-term follow-up study of children who were exposed in utero to routine pelvimetry examinations. Such examinations were carried out in 1948-49 on about 1000 women attending prenatal clinic at Chicago Lying-In Hospital prior to their first delivery, in an effort to determine whether the knowledge gained would lead to easier and more predictable deliveries. Since these examinations were not prompted by medical indication, the exposed children form an ideal group for evaluation of the effects of exposure to diagnostic x-rays. Therefore, a follow-up study of these children was initiated in 1958 by Dr. Melvin Griem. Two control groups were formed, one consisting of children born at Chicago Lying-In Hospital prior to the period of routine pelvimetry, and the other of children born soon after that period. The mothers of these children were contacted by telephone, and information was requested on all significant diseases and hospitalizations. Additional information was obtained from hospital charts.

Preliminary results were published in 1967 [15]. Before evaluating the final results we examined our groups carefully with the intention of identifying possible biasing factors. This led us to limit our analysis to 939 exposed children and 1291 control children who met certain strict guidelines (e.g. all were first-born, received prenatal care at Chicago Lying-In Hospital, and were born there).

When the data were analysed by application of the chi-square test, 10 diseases or disease categories were identified as having an incidence in the pelvimetry group which differed from the incidence in the control groups at the 5% level of significance. Close examination of the data caused us to discount each of these conditions as radiation effects [14]. We clearly identified biasing factors affecting the incidence of two conditions, and there was reason to suspect the presence of such factors for the remaining conditions. Our conclusion was that we could find no convincing evidence for an effect of low-dose radiation, but that our method of follow-up by telephone contact and by abstracting hospital charts did not give reliable results for diseases of a non-serious nature.

Our study could not be expected to shed any light on the incidence of cancer following exposure to diagnostic x-rays. Cancer is rare in children, and for a prospective study a sample many times larger than ours would be required for statistically significant results. Our study is useful, however, in the evaluation of more common conditions, provided that they are of a sufficiently serious nature so that we could accurately determine the true incidence by our method of follow-up.

COMPARISON WITH DIAMOND AND LILIENTFELD'S STUDY

As noted earlier, Diamond and Lilienfeld [9,10] investigated the death rate from all causes and found that the rate among white children who had been exposed in utero to diagnostic x-rays was about double the rate for matched controls who had no history of such exposure. For reasons which remain obscure, they did not find an increased death rate among their black exposed children. Death from any cause is a sufficiently common condition in childhood so that our study, despite its small size, might give statistically significant findings. We therefore compared our results with those of Diamond and Lilienfeld. To make the two studies as comparable as possible, we limited our sample to white single births with survival of at least 28 days, and we limited their sample to children who had been exposed specifically to pelvimetry examinations, and controls matched with these children. The resulting comparison is given in Table III. The relative risk of death following intrauterine irradiation was 1.86 in Diamond and Lilienfeld's study and 0.74 in ours. Using standard statistical techniques [16], we determined that the discrepancy in the results of the two studies (Table III) is significant at the 3% level.

We searched for the cause of this discrepancy by looking closely at those characteristics in which the two studies are dissimilar [14]. Only one characteristic, the method of selection, appeared to be a likely cause. Diamond and Lilienfeld's exposed group, unlike ours, consisted of children exposed in utero to a diagnostic x-ray examination because of some medical indication, such as maternal illness during pregnancy or the suspicion of a pelvis too small to permit normal delivery. As noted in Table II, their exposed group had a significantly higher incidence of complications during pregnancy and of operative delivery than their control group. Table IV demonstrates that, among the

TABLE III. DEATH FROM ALL CAUSES BEFORE AGE 10 AMONG WHITE CHILDREN^a EXPOSED IN UTERO TO PELVIMETRY EXAMINATIONS

	Diamond and Lilienfeld [10]		Oppenheim et al. [14]	
	Exposed	Control	Exposed	Control
Deaths	113.2 ^b	118.3 ^b	9	16
Sample size	7647	14,893	857	1129
Death rate (%)	1.48	0.79	1.05	1.42
Relative risk of death	{ 1.86		{ 0.74	
95% confidence interval	{ 1.43-2.42		{ 0.32-1.70	
Ln rel. risk ± S.E.	0.623±0.131		-0.300±0.414	
Difference ± S.E.	0.922±0.434			
	z=0.922/0.434 = 2.12		p=0.03	

^aExcluding twins and neonatal deaths.

^bBest estimate.

TABLE IV. DEATH AFTER 28 DAYS, RELATED TO MATERNAL COMPLICATIONS OF PREGNANCY AND OPERATIVE DELIVERY
Data of Diamond and Lilienfeld [9]

	Deaths			
	White subjects		Black subjects	
	No./total ^a	Rate (%)	No./total ^a	Rate (%)
Complications of pregnancy				
None	106/10,543	1.01	224/13,175	1.70
Some	83/ 5,056	1.64	194/10,714	1.81
Operative procedures				
None	149/13,241	1.13	377/21,872	1.72
Some	40/ 2,256	1.77	41/ 2,019	2.03
Neither	92/ 9,512	0.97	211/12,696	1.66
Both	26/ 1,228	2.12	28/ 1,541	1.82

^aIncludes exposed and control subjects.

white children in their study, complications during pregnancy and operative delivery were associated with a marked elevation in the childhood death rate. One therefore need not invoke a radiation effect to account for an elevated death rate among these exposed white children.

Although medical indication played no part in the selection process used in our study, we did find that our method of selection introduced an important source of bias in the form of a deficit of premature births in our exposed group. Since prematures are generally less viable than children born at term, this deficit partially accounts for the lower death rate among our exposed children. Its existence can be attributed to the fact that premature delivery decreased the opportunity of obtaining the routine pelvimetry examination. We doubt, however, that this factor can account for the discrepancy between our results and those of Diamond and Lilienfeld, for, although we do not have access to the pertinent data, we suspect that their deficit of prematures among children exposed to pelvimetry examinations is at least as great as ours (we discuss this point in Reference [17]).

COMPARISONS WITH ABCC STUDIES

We are aware of no reported study of routine exposure to diagnostic x-rays other than our own. For additional data on subjects irradiated on a nonselective basis, one must turn to the studies of atomic bomb survivors and their offspring, which are based on data collected by the Atomic Bomb Casualty Commission (ABCC). Kato [18] relates atomic bomb exposure in utero to the subsequent death rate from all causes, for various dose ranges (see Table V). The size of the radiation dose had little effect on the death rate, except perhaps at the highest doses. In fact, Kato's second group had a lower death rate than his first group, although its average dose was presumably 10 or 20 times as great. It is difficult to make comparisons between this post-war Japanese

TABLE V. DEATHS^a BEFORE AGE 10 AMONG CHILDREN EXPOSED IN UTERO TO A-BOMBS, RELATED TO T65 DOSE
Data of Kato [18]

Dose range (rads)	0-9	10-39	40-179	180+	unknown
Median dose (rads)	0	18	72	293	
Sample size	785	222	177	63	26
Deaths	95	23	25	10	2
Death rate (%)	12.1	10.4	14.1	15.9	7.7

^aExcluding perinatal deaths.

TABLE VI. SEX RATIO OF CHILDREN BORN TO MOTHERS IRRADIATED IN UTERO

	X-ray: Meyer et al. [11]				ABCC: Jablon and Kato [19]				
Mother's gestation at exposure (wks)	0-29	30-36	37+	Control	Dose > 10 rads 0-26 27+		Dose < 10 rads 0-26 27+		Control (not in city)
No. male offspring	37	81	122	234	25	18	60	22	52
No. female offspring	18	100	132	254	24	15	55	19	49
Male/female ratio	2.05	0.81	0.92	0.92	1.04	1.20	1.09	1.16	1.06

study and the two American studies discussed above, since the underlying conditions were vastly different and since these conditions strongly influenced the death rates. Nevertheless, if the findings of the Diamond and Lilienfeld study represented real radiation effects, so that an intrauterine dose of the order of one rad was actually responsible for the increased death rate shown in Table III, then one would have expected the much higher doses involved in the ABCC study to have had a stronger influence on the death rate than was actually observed.

Meyer et al. [11] studied the effects of intrauterine exposure to diagnostic x-rays on subsequent offspring of the exposed children. They found a significant excess of males among children whose mothers had been irradiated in utero before 30 weeks of gestation, with a male-to-female ratio of 2.05 (see Table VI). This effect was not confirmed by the ABCC study of Jablon and Kato [19], in which no noticeable effect on the sex ratio of offspring of mothers irradiated in utero was found despite the much higher doses involved (see Table VI). In a later paper [13], Meyer indicated that the trend toward excess male births was reversing itself.

Graham et al. [5] found a significant increase in the risk of leukemia among children whose mothers had had diagnostic x-rays before the children were conceived (see Table VII). The computation of the relative risk was not straightforward in this study, since the authors identified many biasing factors and made numerous adjustments of their data in order to compensate for these factors. The resulting relative risks of leukemia ranged from 1.55 to 1.73,

TABLE VII. LEUKEMIA FOLLOWING PRECONCEPTION IRRADIATION

X-ray: Graham et al. [5]			ABCC: Hoshino et al. [20]		
	Leukemia	Control		Exposed (<2000m)	Control (not in city)
Exposed	N.A.	N.A.	Leukemia cases	2	26
Sample size	313	852	Sample size	14,698	90,872
Exposure rate (%)	46.7 ^a	36.2 ^a	Leukemia rate (%)	0.014	0.029
Relative risk of leukemia	{ 1.60 ^a			{ 0.48	
95% confidence interval	{ 1.18-2.17 ^b			{ 0.11 - 2.06	
Ln rel. risk ± S.E.	0.470±0.152 ^b			-0.743 ± 0.734	
Difference ± S.E.	1.213 ± 0.749				
z = 1.213/0.749 = 1.62 ; p = 0.11					

^aReflect adjustment for year of birth, age of mother, miscarriages or stillbirths.

^bBased on p value of 0.001.

N.A. = not available.

depending on the way in which the data were handled. The computations in Table VII were based on a typical set of values for this study. In order to carry out a statistical analysis of these data, we assumed that the p value for the relative risk was 0.001, which is a more conservative estimate than that given by the authors. We compared this study with the ABCC study of Hoshino et al. [20], in which the rate of incidence of leukemia was determined for children conceived after the bombing. As shown in Table VII, the discrepancy in the results of the two studies has a p value of 0.11, i.e. it is not quite significant at the 10% level. This comparison, however, does not take into account differences in dose. The average radiation dose to the gonads in Graham's study was probably well below 1 rad, since this study included all types of x-ray examinations, only a minority of which involved exposure of the abdomen. In Hoshino's study, the average dose was of the order of 200 rads; yet findings were compatible with no more than a doubling of the leukemia risk (upper 95% confidence limit of 2.06).

Uchida and Curtis [12] found an association between preconception exposure to diagnostic x-rays and mongolism, with a relative risk of mongolism of 4.24; however, the cases and controls were poorly matched. Schull and Neel [21] reported on mongolism in Japanese children conceived after their parents had been exposed to atomic bomb radiation. They found that the risk of mongolism for children whose mothers were present in the city at the time of bombing, relative to the risk for children whose mothers were not in the city, was 0.42. Using standard techniques we found that the difference in the results of the two studies is significant at the 0.001 level (see Table VIII).

One pair of studies is relevant to the association between cancer and intrauterine exposure to diagnostic x-rays. Stewart and Kneale [22] demonstrated a relationship between the number of x-ray films taken during pregnancy and the relative risk of cancer. After appropriate adjustment of the data, they estimated that there would be 572 excess cancer deaths before age 10 for every million person-rads of intrauterine x-ray exposure, with a 95% confidence interval of 306 to 838. Jablon and Kato [23] found only one cancer death among

TABLE VIII. MONGOLISM FOLLOWING PRECONCEPTION IRRADIATION

X-ray: Uchida and Curtis [12]			ABCC: Schull and Neel [21]		
	Mongolism	Control		Exposed (present in city)	Control (not in city)
Exposed	23	13	Mongolism	3	12
Sample size	81	152	Sample size	5582	9452
Exposure rate (%)	28.4	8.6	Mongolism rate (%)	0.054	0.127
Relative risk of mongolism	{ 4.24			{ 0.42	
95% confidence interval	{ 2.00 - 9.00			{ 0.11 - 1.54	
Ln rel. risk \pm S.E.	1.445 \pm 0.375			-0.860 \pm 0.645	
Difference \pm S.E.	2.304 \pm 0.746				
$z = 2.304/0.746 = 3.09$; $p = 0.001$					

699 children who had been exposed in utero to atomic bomb radiation, with the maternal dose ranging from 1 to 499 rads. They gave a value of 17,500 person-rads as a conservative estimate of the accumulated dose for these children. These data correspond to a 95% confidence interval of 0 to 295 excess cancer deaths per million person-rads, which is incompatible with the 95% confidence interval determined by Stewart and Kneale.

SUMMARY

A number of studies appear to demonstrate a causal relationship between intrauterine or preconception exposure to diagnostic x-rays and an increased incidence of leukemia and mongolism, an altered sex ratio, and an increased gross death rate. These studies are subject to criticism on the grounds that radiation exposure was always prompted by some medical indication, so that the irradiated and control groups differed with respect to factors other than the radiation exposure. These factors may have introduced biases into the studies, and these biases, rather than the radiation itself, may be responsible for the observed results. The findings in these studies are not confirmed by others in which radiation exposure occurred on a nonselective basis, from routine pelvimetry examinations or from atomic explosions. This supports the contention that the effects observed in the former studies may be due to biases introduced by the selection process.

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DISCUSSION

Mary E. GAULDEN: Are we correct in assuming that the pelvimetries were performed on your patients late in pregnancy?

I feel that from the standpoint of overall hazards the early stages of gestation, especially the first six weeks, are the most important.

I do not think we can assume that the majority of patients in the Stewart and the MacMahon studies received medically indicated irradiation.

At the period they were exposed (a good many years ago now) pregnant women were often routinely X-rayed.

B.E. OPPENHEIM: The pelvimetric examinations were fairly evenly distributed throughout the second half of pregnancy, only one or two occurring during the first six weeks. On the average, the time of exposure was earlier than in other studies of exposure to diagnostic X rays, in which nearly all examinations took place towards the end of pregnancy. Thus, in terms of time of exposure our study should have been at least as likely to demonstrate radiation effects as the other studies involving diagnostic X rays.

We mean to be very liberal in our definition of a "medically indicated study". As long as a physician submits some but not all of his patients to a pelvimetric examination, we would suspect there are some reasons, perhaps very slight ones, for choosing some patients rather than others. Any such reasons are potential sources of incomparability of the exposed and unexposed groups.

Mary B. MEYER: The statement that in other studies pelvimetry was always medically indicated seems exaggerated because: (a) around 1950, when these studies were performed, pelvimetry was thought to be part of good obstetrical practice and was often done routinely; (b) in the Diamond-Lilienfeld study, 45% of the exposed and 26% of the controls had complications (19% difference). The other 55% of the exposed did not have complications.

Diamond and Lilienfeld found a relative mortality risk of 2 in the "ideal" group (no complications or other risk factors) and in the remainder group.

Comparing relative risks from studies showing non-significant differences with those from studies showing significant differences and finding a significant difference between study results does not seem to be a valid approach.

B.E. OPPENHEIM: We suspect that there may be factors distinguishing Diamond and Lilienfeld's exposed group from their controls even when the samples are restricted to "ideal" groups which are free from certain chosen risk factors. Otherwise, since X-ray exposure was not randomized, why were some subjects exposed and not others?

We certainly feel that it is valid to compare the results of Diamond and Lilienfeld with ours, even though risk differences in our study are not statistically significant. As long as it is valid to estimate the mean and variance of the log relative risk in each study, it is valid to estimate the significance of the difference of means, and this is what we did.

K. SHIMAOKA: We are interested in chronic myelogenous leukaemia (CML) following radiation exposure. We have two cases of CML 18 and 20 years after thymic irradiation, and two cases of CML more than 20 years after pelvimetry. Do you have any CML in your series? Also, was your study cut off at the age of 15, as in the case of Dr. Stewart's?

B.E. OPPENHEIM: We had one or two cases of leukaemia in each of our three groups. The numbers were far too small to attach any significance to cell type.

We contacted our subjects twice. The average age was 15 at the time of the first contact and 19 at the time of the second. We discontinued follow-up in 1970.

M. DELPLA: I am surprised at this discussion — the criticisms made of this excellent study ignore the essential point, which is selection of the irradiated sample. As for doses of one rad or less, I do not really think that there is any possibility of an effect. As we shall see from papers

which will appear in Session IX, much higher doses may even produce a negative added risk.

E.E. POCHIN: Certainly it is important to eliminate the risk of bias in these studies, and it has been suggested that, in the data of Stewart and Kneale, hereditary factors might have increased both the likelihood of maternal X rays (e.g. by affecting pelvic size) and the frequency of malignancies in the children.

It is therefore important to note the estimates that R.H. Mole (Br. J. Cancer 30 (1974) 199) has made on their data. He observed that twins had been exposed to X ray in utero 5.5 times as often as singletons. Despite this high "selection" for X ray, irradiated twins showed no greater incidence of subsequent malignancies, either leukaemia or solid tumours, than singletons.

W.H. ELLETT: You have compared your study population with that observed in Japan. Unfortunately the ABCC¹ study is limited to the years after 1949-50. This makes the data base for in-utero exposures difficult to interpret. How did you correct for the bias due to under-reporting in the Japanese data?

B.E. OPPENHEIM: Kato's study on gross death rate includes deaths occurring during the first five years (1945-1950). Rightly or wrongly, we assumed his data for that period to be accurate.

The incidence of leukaemia mongolism and the sex ratio of offspring might have been influenced by deaths occurring during the early period. We have, however, no way of taking this possibility into account.

¹ Atomic Bomb Casualty Commission.

PROBLEMS ARISING FROM EFFECTS OF LOW RADIATION DOSES IN EARLY PREGNANCY

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Abstract

PROBLEMS ARISING FROM EFFECTS OF LOW RADIATION DOSES IN EARLY PREGNANCY.

From 1970 to 1974, 37 expert opinions concerning interruption of pregnancy because of radiation stress of the foetus had to be given at the Centre for Clinical Radiobiology of the Karl-Marx University, Leipzig. In the cases investigated, radiation stress occurred between the first and tenth week of pregnancy at a maximum amount of ~20 R. X-ray diagnostic measures had been taken in the abdominal range or a tumour radiotherapy had been made, all in cases of unknown pregnancy. The individual determination of the foetal dose showed that different doses result, depending on the setting technique of photographs, physico-technical conditions and body size. In no case did anamnesis show special features. At the given doses an interruption seemed necessary only in one case (radiotherapy). According to this survey, diagnostic radiation stresses in early pregnancy almost always show radiation doses which, at the present stage of knowledge, do not make an interruption seem necessary. Individual differences of dose values for the same exposures, however, make it necessary to calculate exactly the radiation stress of the foetus in each case and to make the corresponding decision. As recent literature agrees in negating a so-called threshold dose for early damage, it is, apart from genetic considerations, necessary to avoid radiation stresses in early pregnancy for this reason also. In the author's opinion, an indication for an interruption is given at doses above 10 R (see also Hammer-Jacobsen, 1959). At lower radiation stresses, an interruption should be recommended in case of simultaneous additional noxae. This paper communicates the results of the check-ups of children who had been subject to a radiation stress of below 7 R in utero in the above cases.

Irradiations of the foetus with appropriate doses mainly induce prenatal death due to resorption at the stage of blastogenesis (pre-implantation period), anomalies of skeleton or organs in the stage of embryogenesis (period of main organ formation) and growth inhibitions or disturbances detectable only histologically or functionally at the stage of the foetal period (growth period). Besides various deformations of the skeletal system and organs, damage to the central nervous system have, most of all, been described. In the investigations of Klingberg et al. [1], retardations of physical development, and especially the development of the electrocorticogram and other central-nervous functions could be proved, e.g. in rats irradiated with 200 R on the 19th day of gestation.

For the human foetus, the 7th to 36th day after conception is claimed as an especially radiosensitive phase [2]. After exposure to sufficiently high doses, the death of the foetus is to be expected mainly within the first three weeks of gravidity, and deformities mainly between the 20th, 36th and 42nd day [3]. Damage to human foetuses has been described repeatedly. The survey of Schaaf [4] up to 1963 covers 107 cases, almost exclusively after therapeutic radiation stresses. Literature on animal experiments shows that the same radiation doses are differently effective on different

days of gravidity, that the effects are dependent on irradiation dose and dose rate and that a threshold dose cannot be detected. In animal experiments, part of the damage during embryogenesis proved to be repairable.

While Henschke [5] and Rajewsky [6] claimed 40 R to be the dose up to which no detectable damage to the foetus should occur at irradiation in the first month, Russell [7] claimed it was 25 R. This value is most quoted in the literature. Already in 1959, however, Hammer-Jacobsen [8] demanded (based on his own observations in man) that an interruption should be recommended in case of foetal stresses in the first month of more than 10 R. This author thinks an interruption of gravidity to be justified even at doses between 1 and 10 R in the presence of additional noxae.

Jacobsen [9] demonstrated in 3150 mouse fetuses that doses of 5 R already induce deformations on the 8th day of gestation in mice (corresponding to the 20th day of pregnancy in man) and does not exclude that these results could be transferred to human relations. Rugh [10] described an increase in mortality and resorption rate of mice by 11% at irradiations with 5 R on the first day of gestation. Despite all problems of transferring such values to relations in man, the conclusion has to be drawn, however, that the question of an interruption of pregnancy at a foetal radiation stress by low doses should be considered carefully case by case. Nokkentved [11] followed up the development of 152 children who had been subject to a radiation stress of between 0.2 R and 7.1 R in the first four months of foetal life. In comparison with a deformation rate of 0.68 - 1.99% in the entire population and with non-stressed brothers and sisters, no increase in anomalies or developmental disturbances could be detected in these children. Only a lower birth weight could be noticed. In that paper, however, dose-effect relations could not be discussed since there were no dose estimates for the individual case.

From 1970 to 1974, 37 expert opinions concerning the interruption of pregnancy because of a radiation stress of the foetus had to be given at the Consultation Centre for Clinical Radiobiology in the Radiological Clinic of the Karl-Marx University, Leipzig. For this purpose a detailed anamnesis as to profession, family and individual case of the married couple was compiled. Special attention was paid to questions about hereditary diseases, deformities in the family, and about noxae which could also induce foetal damage (e.g. drugs, diseases). The dates of conception and of radiation stress during early pregnancy were determined as exactly as possible. Finally, the type of radiation stress was analysed. In case of radiation stresses due to radiodiagnostics, this includes physico-technical exposure conditions and the topographical setting of the shot. In every case we were shown the original photographs. If, in radiodiagnostic examinations, the uterus lay in the cone of the useful beam, radiation stress was estimated according to Heller et al. [12]. Allowing for all necessary parameters and correcting factors, the dose was determined in terms of mR/mA·s. If the stress was due only to scattered radiation, the dose was estimated by our own measurements for all radiodiagnostic measures in the trunk. In doing so, the area-dose product calculated afterwards and the distance of the central ray from the uterus were allowed for.

The expert opinions have been listed in Table I. In most cases the application for interruption was made to the gynaecologist by the married couple themselves. The radiation stress occurred between the first and tenth week of pregnancy. It amounted maximally to ~20 R. Radiodiagnostic

TABLE I. EXPERT OPINIONS ON RADIATION STRESSES IN EARLY GRAVIDITY

Serial no.	Age Female	Male	Anamnesis	Time of radiation stress	Type of radiation stress : Indication	Radiation dose (foetus)	Inter-ruption
1	33	36	Without findings	2nd - 3rd week	Lumbar spine (with pelvis): spondylosis	~ 1 R	No
2	30	32	Ovarectomy, right, 4 years ago; fertility op. 1 year ago	~ 16th day	Lumbar spine and dorsolumbar transit., 2 levels: backache	~ 0.65 R	No
3	31	37	Without findings	6th week	Peroral cholecystogram: bilious complaint	< 0.2 R	No
4	26	30	Radioiodine test, 1 year earlier	15th - 20th day	Colon contrastive agent: vague abdominal pains	~ 2.5 R	No
5	28	35	Without findings	4th week	Lumbar spine 2 levels (without pelvis)	~ 0.2 R	No
6	24	23	Without findings	1st - 4th week	Radiodiagnostic work in practical term, 16 d (1.5 h each) X ray: stomach, thorax, with radiation protection, no dose meter	< 1 R/ 4 weeks (control measurements)	No
7	27	26	Chronic adnexitis on the right	1st - 4th week	X ray at image converter at op. (6x5 min and 1x45 min with protection; 45 min without protection), no dose meter	< 1 R (work without protection)	No
8	41	44	Without findings	2nd - 3rd week	Thorax, 2 levels, peroral cholecystogram (7 exposures): gall-stones	< 2 R	No
9	27	27	Without findings	3rd week	Lumbar spine, 2 levels with pelvis (5 exposures): backache	~ 1 R	No
10	21	24	Without findings	2nd week	i.v. pyelogram (4 exposures): backache	1 - 2 R	No
11	18	20	Without findings	3rd week	i.v. pyelogram (2 exposures): renal complaint	1 - 2 R	No
12	24	25	Without findings	2nd - 3rd week	i.v. pyelogram (4 exposures): nephritic stone	1 - 2 R	No
13	23	24	Without findings	6th week	X ray of stomach: nausea	1 R	No
14	23	27	Without findings	7th week	X ray of stomach: chronic stomach trouble	1 R	No

TABLE I. (cont.)

Serial no.	Age		Anamnesis	Time of radiation stress	Type of radiation stress ; Indication	Radiation dose (foetus)	Inter-ruption
	Female	Male					
15	22	21	Without findings	4th week	G-i tract: obstipation	2 R	No
16	19	22	Without findings	9th week	X ray of thorax, routine examination	0.1 R	No
17	27	33	Without findings	2nd week	i.v. pyelogram: backache	3 - 5 R	No
18	35	38	Without findings	3rd - 5th week	X ray of stomach and gall: epigastric complaint	~1 R	No
19	24	24	Without findings	3rd week	Aortography, renal anamnesis	3.5 R	No
20	31	38	Without findings	6th week	X ray routine examination	0.2 R	No
21	31	30	Without findings	3rd week	X ray of pelvis: static complaint	0.5 R	No
22	27	29	Without findings	4th - 6th week	Cholecystogram: epigastric complaint	1 R	No
23	19	18	Without findings	8th week	X ray of stomach: epigastric complaint	1 R	Yes, on application of patient
24	35	42	Without findings	10th week	X ray of stomach and thorax: epigastric complaint	3 R	No
25	36	36	Without findings	8th week	X ray of thorax, stomach and gall: epigastric complaint	2 R	No
26	27	28	Without findings	6th week	Lumbar spine 2 levels, pelvis, i.v. pyelogram: epigastric complaint	6.8 R	No
27	22	28	Without findings	6th week	Right ankle joint, 2 levels: sprain	0.1 R	Yes, on application of patient
28	22	21	Without findings	3rd - 4th week	X ray of stomach and gall: epigastric pains for 2 years	0.1 R	No
29	25	29	Without findings	5th - 8th week	X ray of stomach and gall	0.2 R	No
30	26	29	Without findings	4th week	Arteriography of pelvis and legs: accident	1 R	No
31	29	34	Without findings	6th week	X ray of stomach: epigastric complaint	0.2 R	No

TABLE I (cont.)

Serial no.	Age Female	Male	Anamnesis	Time of radiation stress	Type of radiation stress: Indication	Radiation dose (foetus)	Interruption
32	24	22	Without findings	2nd week	X ray of stomach: ulcer suspected	0.1 R	No
33	21	21	Without findings	1st week	X ray of gall: epigastric complaint	0.2 R	No
34	23	24	Professional radiation exposure (X-ray assistant)	1st - 6th week	Radiodiagnostic work	0.01 R	No
35	18	20	Without findings	4th week	X ray of pelvis: backache	0.2 R	No
36	19	24	Without findings	1st week	Arms, skull, pelvis: accident	1 R	No
37	21	23	Without findings	1st week	Telecobalt therapy: Hodgkin's disease	~20 R	No

measures had been taken in the abdominal range or a tumour therapy had been made (1 case), all in cases of unknown pregnancy. In three cases there were professional radiation exposures. In ten cases (examination after the 6th week) it must be stated that a gravidity should have been assumed in the absence of menstruation. In almost all cases, the indication for X-ray examination had been followed with due care. Possibilities for gonad protection, however, were not always made use of. The individual calculation of the foetal dose shows that different doses result from the setting technique of photographs, physico-technical conditions and body size. Anamnesis in no case showed special features. At the given doses, an interruption seemed necessary only in one case (radiotherapeutic measures during early gravidity). In two other cases the interruption was performed on application of patients for fear of radiation damage, despite proper information. In personal talks, the married couples' attention was drawn to the natural rate of deformations in general. The three cases of professional radiation exposure showed a neglect of instructions for radiation protection, but the foetal stress was below 1 R.

In 15 cases check-ups were possible of the children born who had been subject to a radiation stress of below 7 R in utero in the above group (Table II). According to the characteristics of the material investigated, these check-ups were made within a period of 1 - 3 years after birth. The results are as follows. The process of pregnancies was normal. Weight and body size at the time of birth were within the range of reference. There were all indications of full development at the time of birth. Physical and mental development of all children was normal within the period of investigation. There were no chronic disturbances of health. There were no differences between them and brothers or sisters. In one case (No. 22) the baby had a talipes calcaneus at birth, which was corrected by orthopaedic treatment.

TABLE II. CHECK-UPS OF CHILDREN BORN FROM CASES SHOWN IN TABLE I

Serial No. ^a	Brothers /sisters	Course of gravity	Weight and size at birth	Fully developed at birth	Deformity	Physical and mental development (1st-3rd year)	Chronic disturbances
1	♀ normal, 10 years earlier	Without findings	Normal	Yes	No	Normal	No
2	Extra-uterine gravity, 4 years earlier	Without findings	Normal	Yes	No	Normal	No
4	♂ normal, 1 year earlier	Without findings	Normal	Yes	No	Normal	No
9	♂ spina bifida, 5 years earlier	Without findings	Normal	Yes	No	Normal	No
17	None	Premature birth, insufficiency of placenta	2460 g 47 cm	No	Myelo-meningocele lumbo-sacralis, talipedes calcanei, struma	Normal	No
18	None	Without findings	Normal	Yes	No	Normal	No
19	None	Without findings	Normal	Yes	No	Normal	No
20	None	Without findings	Normal	Yes	No	Normal	No
21	None	Without findings	Normal	Yes	No	Normal	No
22	None	Without findings	Normal	Yes	Talipes calcaneus	Normal	No
24	♀ 16 years earlier, ♂ 12 years earlier, normal	Without findings	Normal	Yes	No	Normal	No
26	♂ 8 years earlier, ♀ 5 years earlier, ♂ 4 years earlier, normal	Without findings	Normal	Yes	No	Normal	No

TABLE II (cont.)

Serial No. ^a	Brothers /sisters	Course of gravidity	Weight and size at birth	Fully developed at birth	Deformity	Physical and mental development (1st-3rd year)	Chronic disturbances
28	None	Without findings	Normal	Yes	No	Normal	No
29	♀ normal, 3 years earlier	Without findings	Normal	Yes	No	Normal	No
33	None	Without findings	Normal	Yes	No	Normal	No

^a Serial Nos. refer to those of Table I.

Foetal stress was less than 1 R. A causal relation must be excluded. Another case (No. 17) after foetal stress of between 3 and 5 R in the second week of pregnancy is remarkable. Here there was a premature birth with myelomeningocele lumbosacralis and talipes calcaneus corrected later on. The question of how far this deformation was radiation-induced cannot be answered. In view of dose amount and type of deformation, however, a radiation influence seems to be less probable. The given material investigated does not allow any further differentiation, so that its interpretation must remain limited. For this clinical study, a control group could not be formed.

A problem interesting for reasons of comparison is that of conception after previous radiation stresses of the testes. The radiosensitivity of the stages of spermiogenesis is different. For this reason so-called early conceptions are possible within the first 3 - 4 weeks after irradiations of testes. The developmental stage between spermatocytes and spermatids is claimed to be especially sensitive to dominant lethal mutations (e.g. Ref. [13]). In animal experiments, early conceptions were characterized above all by an increase in abortions [14]. Due to dominant point mutations, however, skeletal anomalies are also possible [15]. Researchers therefore agree in seeing a great risk in early conceptions. Late conceptions a longer time after testes irradiation are possible even after exposure to high-dose radiation, since there may be a repair by surviving spermatogonia. In animal tests they less often induce damages in the F_1 generation (Langendorff [16]), but in rats, apart from deformities, decreased vitality and life expectancy as well as decreased intelligence have, above all, been described [14]. Up to date, there have been hardly any systematic investigations of high-dose irradiations of human testes.

There are comparable problems in conceptions after irradiations of ovaries. In contrast to fully developed follicles, the early oocyte and intermediary stages are especially radiosensitive [13-16]. In single cases, surviving oocytes can make conception possible even after high-dose irradiations of ovaries. Among possible damages in the F_1 generation, foetal death due to dominant lethal mutations predominates [14]. The probability that oocytes transmit radiation-induced mutations on viable progeny is claimed by Langendorff [16] to be limited since, out of the large number of oocytes,

TABLE III. APPLICATIONS FOR INTERRUPTION AFTER RADIATION STRESSES OF TESTES

Serial no.	Age Female	Male	Anamnesis	Time of testes irradiation before conception	Type of radiation stress : Indication	Radiation dose (testes)	Interruption
1	32	38	Male: without findings; female: German measles in early gravidity	9 years	Radiotherapy at seminoma on the right	Left testis ~1400 R /6 weeks	Yes
2	36	36	Male: without findings; female: T.B. 2 years earlier	5 years	Radiotherapy at seminoma on the right	Left testis ~1300 R /5 weeks	Yes
3	29	33	Without findings	3 years	Radiotherapy at seminoma on the left	Right testis ~1200 R /5 weeks	Yes
4	18	19	Without findings	During conception	Cobalt therapy: melanoma on left side of neck	Testes < 1 R. /2 weeks	No

only 350 - 400 cells will mature during puberty and only very few will be impregnated. References [17 - 23] indicate the possibility of damage to the F_1 generation after radiation stresses of ovaries. This is supported by the increased number of abortions and the sex reversals. Reliable statements on an increased deformation rate, however, do not yet seem to be possible. It is therefore necessary, also in the case of radiation stress of parents' gonads, to check carefully the question of an interruption of pregnancy in each case.

The radiation stress of gonads after previous radiotherapy is estimated by allowing for the position of gonads relative to irradiation zone according to tables of depth doses, isodose curves and our own measurements, especially concerning the loss along the field boundary of direct exposure.

Table III gives a survey on applications for interruptions after radiation stresses on testes. In cases 1 to 3 there was a radiation stress of the remaining testis with 1200 to 1400 R for 5 - 6 weeks due to seminoma irradiations which dated back several years. All three patients claimed that after radiotherapy they had suffered from a decrease in libido and potency for one up to maximally five years. Afterwards sexual functions normalized again. In these three cases there was a so-called late impregnation. The sperms inducing conception came from radiation-stressed spermatogonia. As communications in the present literature do not exclude possible damages in the F_1 generation, in all three cases we applied for an interruption of pregnancy in view of the given high radiation stress of the testis. In case no. 1, in addition, the patient had contracted German measles during early gravidity. In case no. 4 there was a negligible radiation stress of the testes.

TABLE IV. RADIATION STRESS OF OVARIES

Serial no.	Age Female Male	Anamnesis	Time of ovary irradiation before conception	Type of radiation stress : Indication	Radiation dose (ovary)	Interruption
1	35 38	Without findings	11 years	Radiotherapy: fibrosarcoma at left groin	Left ovary ~3 R, /4 weeks	No
2	18 21	Without findings	12 years	Radiotherapy, maxillary cavity: carcinoma on the right	Both ovaries <1 R, /6 weeks	No
3	29 34	Without findings	1 year	Cobalt therapy, thyroid gland: carcinoma	Both ovaries ~0.1 R, /4 weeks	No
4	31 36	Without findings	29 years	Radiotherapy, kidney: carcinoma on the right	Both ovaries ~4 R /3 weeks	No

TABLE V. CHECK-UP OF CHILDREN BORN FROM CASES SHOWN IN TABLE IV

Serial No. ^a	Brothers /sisters	Course of gravidity	Weight and size at birth	Fully developed at birth	Deformity	Physical and mental development (1st-3rd year)	Chronic disturbances
1	None	Without findings	Normal	Yes	No	Normal	No
2	♂normal 1 year later	Without findings	Normal	Yes	No	Normal	No
3	None	Without findings	Normal	Yes	No	Normal	No

^a Serial Nos. refer to those of Table IV.

Table IV shows four cases of late impregnations after low radiation stresses of one or both ovaries, which did not justify an interruption. They showed, however, that highly different radiation doses and/or situations have to be expected in medical advice on interruptions. The check-ups of children (Table V) did not yield any special characteristics.

Summing up, the following conclusions may be drawn from our clinical study:

(1) According to our survey, in diagnostic radiation stresses during early pregnancy the radiation doses do not in most cases make an interruption necessary in the light of our present knowledge. Individual differences in values at the same exposures, however, call for an exact calculation of

- foetal radiation stress in each case and an appropriate decision. As recent literature agrees in rejecting a so-called threshold dose for early damage, it is necessary in any case to avoid radiation stresses during early gravidity. The rule to make X-ray examinations of the female abdomen only within the first week following menstruation should be paid still more attention. Thus irradiations of unknown pregnancies could be avoided, as found in all our cases.
- (2) Our check-ups of children irradiated in utero confirm the correctness of the above statement. On the borderline of radiation stress with low doses during early gravidity it can barely be decided if, in case of deformities, these are induced by radiation. In our opinion an indication for an interruption is given at foetal stresses of above 10 R (see also Hammer-Jacobsen [8]). At lower radiation stresses, an interruption should be recommended in the presence of additional noxae.
- (3) At radiation stresses of testes during radiotherapeutic measures, the patient must in every case be informed that conception has to be avoided. This holds especially for early impregnations. The use of ovulation inhibitors for contraception can be recommended to wives. This should also hold for avoiding late impregnations.
- (4) Expert opinions or medical advice on interruptions of pregnancy for radiobiological reasons should be given only by a doctor with special radiobiological knowledge in co-operation with a clinical radiophysicist. In our expertises, anamnesis showed in almost all cases that the applicants had been extremely frightened by gynaecologists, general practitioners and radiologists about potential radiation damage to the child. Therefore our refusal of interruption was not appreciated by any of the patients and made informative talks necessary.
- (5) Extensive epidemiological, radiobiological and radioprotective medical investigations will be necessary to arrive at final proposals and decisions in the practically important range of problems of the biological effect of low-radiation doses.

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DISCUSSION

Mary E. GAULDEN: I applaud your plans to perform chromosome studies on future cases. It is most important to follow these children for long periods of time, because the effects of diagnostic radiation doses are more likely to be subtle than gross effects detectable at birth. The induction of mosaics with one or more chromosome abnormalities is an expected effect of irradiation, especially from exposure during early development (first six weeks). That chromosome mosaics can arise in human embryos is suggested by several studies on abortuses induced after diagnostic radiation exposure. Data on Dawn's syndrome patients who are mosaics show that those with few and/or mild physical stigmata can exhibit pronounced mental retardation. None of the animal studies on effects of radiation on embryos gives us any information on cognitive ability; there is abundant evidence in the human that small chromosome abnormalities contribute to significant defects in the central nervous system. Thus a radiation-induced chromosomal mosaic may suffer reduced defects of the central nervous system which might not be detectable at birth.

In my practice, I have noted a tendency of physicians to order a large number of X-ray examinations in the absence of acute or emergency conditions. In many cases, the only problem the patient is revealed to have is early pregnancy. Better education of physicians regarding the risks is mandatory. Elective scheduling reduces exposure of unsuspected conceptuses.

LONG-TERM EFFECTS OF PRENATAL X RAY ON DEVELOPMENT AND FERTILITY OF HUMAN FEMALES*

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Abstract

LONG-TERM EFFECTS OF PRENATAL X RAY ON DEVELOPMENT AND FERTILITY OF HUMAN FEMALES.

This continuing epidemiologic study investigates possible effects of X-ray exposure during foetal life on the subsequent development and fertility of human females and on their offspring. The study population comprises 1458 females exposed in utero to maternal diagnostic X ray such as pelvimetry, and 1458 unexposed controls matched by hospital of birth, parity, race and birthdate (1947-1952). Phase I of the study showed a 10-15% increase in fertility in young exposed women, based on ascertainment of live births and foetal deaths registered in Baltimore City. This statistically significant difference ($p = 0.011$) remained after adjustment for differences between exposed and control women in economic, social and medical factors. Phase II of the study, a direct follow-up of exposed control pairs of women now aged 22-28, continues to show more total pregnancies among exposed women. Exposed-in-utero women had 15% more total pregnancies than controls in 1960-1969, and 7% more in 1970-75. Exposed and control women are similar in number of siblings, number of children wanted, contraceptive use, and frequency of therapeutic abortion. Other findings suggest possible exposed-control differences in growth, development and behaviour. Exposed women have completed fewer grades of school, have poorer general health, more menstrual problems, more of certain diseases and accidents, and are heavier for height than controls.

Introduction

The purpose of the long-term study to be reported here is to investigate the possible effects of in-utero exposure to diagnostic x-rays on the later development and fertility of human females, and on their children in turn. Our reasons for undertaking this study ten years ago were both theoretical and practical. In the theoretical area, experimental studies in a number of species have shown that female germ cells have two periods of extreme sensitivity to radiation damage, the first coinciding with peak activity of oogonia, especially the last premeiotic division, the second with entry of the cells into the diplotene or dictyate stage (1-7). In the human both of these stages are probably completed during fetal life, and the human female is born with a finite supply of potential ova (8,9). Although oogenesis and radiation effects in different species follow a reasonably consistent pattern, it is impossible to extrapolate from one species to another in any detail, and conclusions about effects in humans must be derived from human studies. Recognition of the teratogenic effects of prenatal x-ray in early pregnancy has led to postponement of pelvimetry to late in gestation, possibly to a more dangerous time for the fetal gonads. Prenatal damage of the germ cells would be expected

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to show up in later reproductive performance, and could be revealed by a well-designed epidemiologic study. Practical considerations were that a previous study had identified a large group of children who had been exposed to x-rays during fetal life, had selected matched, unexposed controls, and had followed up this large population during childhood. Population and data from this study were available to us, and ascertainment of births to a selected segment of the females proved to be feasible.

Study population and methods

Pelvimetry-leukemia study. The original study, known as the Pelvimetry-leukemia study, was designed to test the effect of prenatal diagnostic x-ray on survival of the offspring. Births from pregnancies with any abdominal x-ray exposure were ascertained in nine Baltimore hospitals for the years 1947-1956, and each was matched with two unexposed births by race, sex, hospital, birth order and birthdate (Table I). A search for these 45 000 children was carried out in 1961-1967 to ascertain survival status, and cause of death for non-survivors. Ninety-five percent of these children were found, and 85-90% of the black population were found still to be living in Baltimore City. Analysis of results showed that among whites the mortality of the exposed group during the first ten years of life was almost twice that of controls, whereas among blacks there was no mortality difference by exposure (10).

TABLE I. INTERRELATIONSHIP OF TWO LINKED STUDIES

Name: PELVIMETRY-LEUKEMIA		PELVIMETRY F ₂	
		PHASE I	PHASE II
Date done: 1961-1967		1965-1972	1973-present
Population			
P ₁	<u>PREGNANT WOMEN</u>		
	20 000 x-rayed		
	36 000 controls*		
	9 Baltimore hospitals		
	↓		
F ₁	<u>BABIES</u>	<u>POTENTIAL MOTHERS</u>	<u>YOUNG WOMEN</u>
	Exposed in utero	1458 Exposed	Direct follow-up
	Controls, matched*	1458 Controls*	Growth, development,
	Born 1947-1956	Born 1947-1952	health, fertility,
		Black females	personal, social
		Baltimore residents	↓
F ₂		<u>BIRTHS</u>	<u>PREGNANCIES</u>
		Live births	Questionnaire data
		Fetal deaths	Child information
		Baltimore 1960-1972	

*Matched by race, sex, parity, hospital, date of birth.

Pelvimetry F_2 study. The subject of this report, the Pelvimetry F_2 study, was designed to test the hypothesis that female ovaries and germ cells exposed to low doses of x-ray during fetal life may be affected in ways measurable in terms of later reproductive experience. The relationship between this and the original study population is shown in Table I. Our population is a subset of the original one, consisting of black exposed and control females who were born in the years 1947-1952 and were found in Baltimore City schools at the time of follow-up in the 1960's. Black women were selected because they start having babies earlier than white women, and because all were Baltimore residents who would have their births registered in Baltimore City. We have therefore selected survivors healthy enough to be in school, in sets of matched pairs. Each exposed woman has an unexposed partner who was born at the same hospital, of the same birth order, and who is the same age, often to the exact day of birth. The matched-pair design of the study is crucial to its success, particularly in view of the strong relationship between age and reproductive rate. It also allows retrospective matching on additional factors, as will be demonstrated.

The majority of x-ray exposures were for pelvimetry; other types included placentograms, flat plates, and a few procedures not related to pregnancy. Previous analysis indicated that 80-85% were done as a part of routine obstetrical care rather than for indicated medical reasons (11). Dosage to the whole fetus is estimated to be in the range of 1-5 rads in most cases, based on the number of successful films recorded and estimates of dosages per film for different procedures. These dosage data are probably inadequate for dose-response analysis. However, the time of x-ray is known for 87% of the population, and experimental findings indicate that time of exposure has a stronger influence on outcome than do small differences in dosage. Most x-rays were done in the third trimester, with the peak number at 37.5 weeks (figure 1). Thirty-one percent were done on the day of delivery.

PHASE I: Ascertainment of F_2 births

The primary purpose of the first phase of the study was to test the study hypothesis that fetal ovaries and oocytes would be affected by x-ray exposure, by comparing reproductive patterns in exposed and control

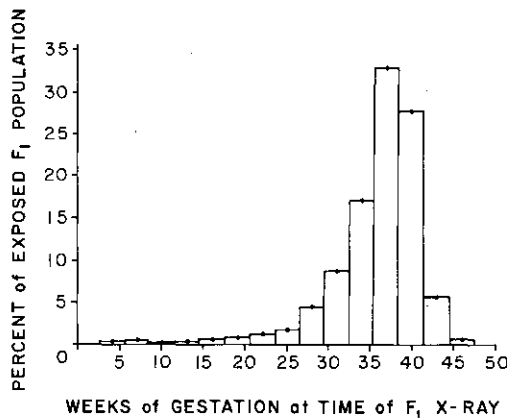


FIG.1. Distribution of X rays by week of gestation.

women. To accomplish this, our aim was to ascertain all live births and fetal deaths for the 1458 matched pairs of black female residents of Baltimore City who comprise the study population. This was done by matching maiden names, obtained from Baltimore birth certificates for women of appropriate age, race and birthplace, with those of study population. Birth certificate information for all births, and hospital records for a sample of mothers and babies, were abstracted. Characteristics of births to exposed and control mothers were compared for differences possibly related to prenatal x-ray exposure, including overall pregnancy rates, complications of pregnancy and labor, age at first birth, fetal and neonatal loss, cause of death, malformations, and the like. We also investigated possible indirect relationships, realizing that maternal conditions in the P_1 generation that were indications for pelvimetry or other diagnostic x-ray might be passed on to the F_1 daughters, causing differences between them and the unexposed controls in reproductive success or failure. This analysis showed little correlation between generations of births for these conditions (11).

Results

Births occurring through 1971 have been ascertained and analysed. At the time of analysis, we had ascertained a total of 2368 live births with 94 neonatal or later deaths, and 54 fetal deaths, among our listed population of 2916 women whose ages in 1971 ranged from 19 to 25 (Table II). The most outstanding difference between exposed and control mothers was that exposed mothers had significantly more registered births than controls, including almost twice as many fetal deaths. Deaths among live births were not significantly different in the two groups, the rate per 100 live births being slightly higher among controls. (Abortions were not ascertained in Phase I of the study.)

Table II. F_2 births and mortality ascertained through 1971, by exposure status.

	<u>Exposed</u>		<u>Control</u>		<u>P</u>	
	<u>Number</u>	<u>Rate</u>	<u>Number</u>	<u>Rate</u>	<u>χ^2*</u>	<u>P</u>
Live births and fetal deaths	1482	102 ^a	1320	90 ^a	9.3	.002
Live births	1445	99 ^a	1300	89 ^a	7.6	.006
Deaths among live births	53	37 ^b	57	44 ^b	0.6	N.S.
Fetal deaths	37	24 ^c	20	15 ^c	4.5	.03

a per 100 F_1 females.

b per 1000 live births.

c per 1000 total births.

* χ^2 with 1 d.f. corrected for continuity.

Finding increased fertility among women who had been exposed to x-ray in utero was unexpected, and part of the thrust of our analysis has been to test the possibility that the fertility difference might be due to differences between the two groups unrelated or only indirectly related to x-ray exposure. For example, higher fertility rates were found among F_1 women whose mothers had been unmarried when they were born, or who were of low economic status as indicated by hospital pay status (welfare, ward, or private), or by census tract of residence. X-ray exposure was more frequent among welfare patients, and slightly more frequent among unmarried women. Reported complications, especially contracted pelvis and cephalo-pelvic disproportion, were more frequent in the exposed group for obvious reasons, but there was no relationship between the presence of these complications in the first generation and fertility patterns in the next.

We have used three analytic approaches to test whether the observed fertility differences between exposed and control F_1 women may be due to socioeconomic factors rather than to radiation exposure (12).

First, exposed and control fertility rates were compared within subgroups according to all factors related to fertility and unequally distributed between exposure groups. In every case pregnancy rates were higher for exposed than for control women, the differences being greater at higher socioeconomic levels. Our second analytic approach to the same question was to add more matching factors to the original ones, a process made possible by the pair-matched structure of the

Table III. Fertility of exposed and control F_1 population with added matching factors, by economic level of residence

Added matching factors	Number of F_1 pairs	Economic level	Fertility rate F_2 births and fetal deaths per 100 F_1	
			Exposed	Control
None	1453	1-2	110	103
		3	93	88
		4-5	73	62
P_1 hospital status	917	1-2	109	104
		3	87	74
		4-5	77	64
P_1 hospital status and		1-2	123	111
P_1 complications and	319	3	85	60
P_1 marital status		4-5	66	55

original study. Enough information was available, and the study population and number of births were large enough to allow this technique to be used. The results of this process are shown in Table III. The higher fertility rates of exposed mothers are still present even when the matching factors of P_1 hospital status, marital status, complications, and the economic level of F_1 residence are added, based on 319 super-matched pairs. A third analytic approach was a simultaneous multi-factor adjustment of the fertility rates for each factor found to be important in relation to fertility and to be unevenly distributed between exposed and control groups, using a multiple linear regression model. Exposed women had 11% more registered births than controls, and the adjusted fertility difference between exposed and control F_1 women has a p value of .011. The pattern of higher fertility among exposed F_1 women is consistently observed in all subgroups, and is relatively greater at the higher socioeconomic levels (12).

Phase II: Direct follow-up of F_1 women

None of the analytic methods used in Phase I had explained the fertility difference, and as the study continued it became increasingly clear that a number of questions remained that could only be answered by direct contact with the F_1 women. For example, were similar proportions of exposed and control women still living in Baltimore? Did the two groups differ in such characteristics as size of their original family, in the number of children wanted, in contraceptive use, and the like? Other advantages of direct contact with the study subject included the possibility of determining differences in these young adult women themselves that might be late manifestations of their prenatal x-ray exposure. Findings from animal and human studies suggest that these might include growth retardation, mental retardation, hormonal changes manifested as menstrual problems and ovarian cysts, differences in general health and longevity as well as in particular diseases, and effects on the brain and nervous system manifested by changes in behavior and personality (13-24).

Phase II Methods: Preliminary findings from Phase II reported here are based on follow-up of 824 pairs of exposed and control women matched on the original factors and on economic level. Questionnaires were mailed in 1974-75 to the 1555 of these 1648 women who were found to be alive and for whom current addresses could be located. Similar proportions of the exposed and control groups were found to be deceased, could not be traced, or refused to answer. In these categories 1.5% of the exposed and 1% of the controls had died in recent years, and 4% of each group could not be traced. Equal proportions of the two groups had remained in Baltimore. Of those who received questionnaires, 83% completed them either spontaneously or after telephone or personal follow-up, 13% of exposed and 12% of controls refused, and in 4% and 5% of the two groups follow-up was abandoned after repeated attempts to obtain answers.

Fertility: Pregnancies reported on questionnaires by exposed and control women are compared in Table IV. Fewer exposed women are in the never pregnant category. Exposed women have had more total pregnancies than control women, including more live births, almost twice as many fetal deaths, more spontaneous abortions, and the same number of induced abortions. They have had more births at all parities, indicating that those with any births have had larger families. By calendar time, the excess of exposed over control for total pregnancies is 15% in the years 1960-1969, and 7% in the years 1970-1975. For fetal deaths and spontaneous abortions, exposed women had 18% more than controls in 1960-69, and 33% more in 1970-75.

Table IV. Pregnancy data from questionnaire follow-up of exposed and control F₁ women 1974-1975

A. Pregnancies by exposure and outcome

	<u>Exposed</u>	<u>Control</u>	χ^2 1 df.	<u>P*</u>
Responses	643	648		
Never pregnant	132	160	2.3	N.S.
Pregnancies: Total	1106	1001	4.9	.03
Born alive	884	800	4.8	.03
Deaths	22	23		N.S.
Fetal deaths	25	13	3.3	N.S.
Spontaneous abortions	87	76	0.7	N.S.
Induced abortions	103	103	0.0	N.S.

B. Pregnancies by exposure and birth order

Pregnancy number:				
First	511	489	0.6	N.S.
Second	308	286	0.9	N.S.
Third or fourth	244	192	6.3	.02
Fifth or more	43	34	1.0	N.S.

C. Pregnancies by exposure and calendar time

Total pregnancies:				
1960-1969	559	487	5.5	.02
1970-1975	528	494	1.4	N.S.
Fetal deaths and spontaneous abortions:				
1960-1969	52	44	0.6	N.S.
1970-1975	56	42	1.8	N.S.

χ^2 with 1 d.f. corrected for continuity. Two-tailed test.

*Failure of totals to agree is due to omission of unknowns.

Non-radiation factors that might affect fertility are compared in Table V. Exposed women do not come from larger families or want more children than controls. There are no significant differences in the proportions of exposed and control women who have had unwanted pregnancies, in birth control use or success, or in the frequency of therapeutic abortions reported. Significantly more exposed women have been sterilized, and the rate of sterilization rises dramatically with parity, a fact that may make it difficult to find out whether fertility declines naturally more rapidly in exposed than in control women in this study (figure 2).

Table V. Other factors possibly related to F_1 fertility, by exposure. Questionnaire data 1974-1975.

<u>Description</u>	N= 643	N= 648	χ^2 1 d.f.
	<u>Exposed</u> %*	<u>Control</u> %*	
Size of F_1 family (F_1 and sibs):			
Five or more	47.5	50.5	
Number of children wanted:			
None, one or two	58.0	58.4	
Unwanted pregnancies:			
One	36.7	37.6	
Two or more	16.7	13.7	1.6
Birth control used now:			
Sometimes, usually	10.3	10.1	
Always	61.4	63.4	
Birth control methods ever used:			
Pill: used	68.9	73.0	
did not work	11.5	8.9	1.3
IUD: used	26.9	26.4	
did not work	18.5	21.6	
All others (combined): used	57.1	51.7	
did not work	28.6	21.7	3.0
Marital status:			
Married	38.6	32.6	2.9
Sterilized	9.6	6.2	4.3 ⁺

*Percent of total responding.

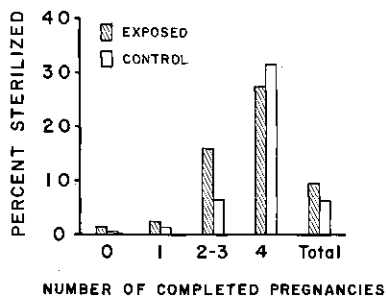
⁺ p = .04. No significant differences in other comparisons.

FIG.2. Sterilizations by exposure and number of completed pregnancies (questionnaire data).

Table VI. Factors related to growth, development, and health by exposure. Questionnaire data 1974-1975

Description	N= 643 Exposed %*	N= 648 Control %*	χ^2 1 df ⁺	P
Grades of school completed:				
Eleven or less	32.7	27.2	3.4	N.S.
Reason for leaving school:				
Graduated or still attending	54.3	59.7	1.4	N.S.
Pregnancy	19.8	14.5	5.2	.03
Physical or mental disability	1.6	0.8	1.1	N.S.
General health now:				
Good or excellent	63.6	68.5	1.1	N.S.
Fair or poor	19.9	15.7	2.9	N.S.
Menstrual problems requiring medical attention:				
Yes	29.4	21.4	4.8	.03
Height, inches:				
62 or less	35.2	26.3	8.0	.005
66 or more	29.2	38.4	7.7	.006
Weight, pounds:				
160+	23.6	19.0	2.9	N.S.
Ponderal index:				
<11 (heavy for height)	17.4	10.8	6.5	.01

*Percent of total responses.

⁺ χ^2 with 1 d.f. corrected for continuity. 2-tailed test.F₁ health and development

Table VI shows questionnaire results related to growth, health and development of the F₁ women. Exposed women have finished fewer grades of school, fewer have graduated, and twice as many exposed as controls have left for reasons of physical or mental disability. Significantly more exposed women have left school because of pregnancy.

Exposed women are significantly shorter in stature than controls, with no difference in weight distribution. They therefore have significantly lower ponderal indices (heavy for height). As one would expect, more of them regard themselves as too fat. Preliminary data suggest that some part of the height difference in F₁ women is due to the increased probability of x-ray pelvimetry in F₁ mothers of short stature. General health is poorer among the exposed women, and significantly more of them have sought medical help for menstrual problems. Questionnaire responses to a list of diseases and accidents showed the overall incidence to be higher for the exposed group, due to increased

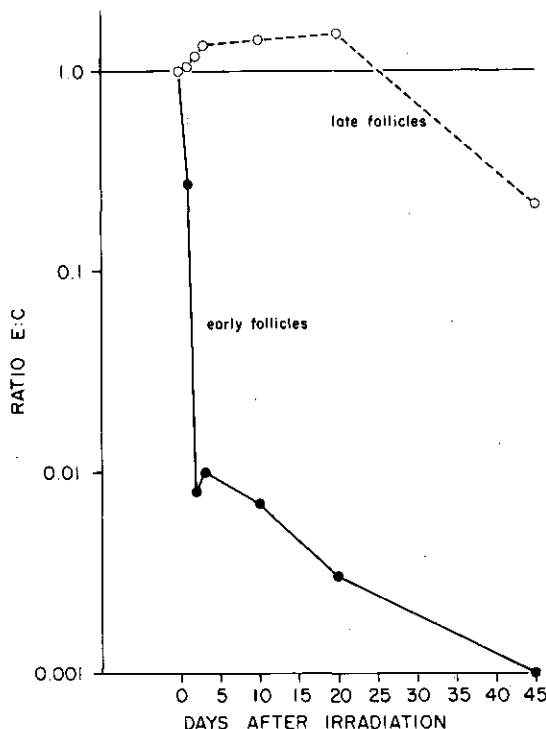


FIG.3. Survival of mouse oocytes exposed to 50 R, for early and late follicles, by time after exposure (from Oakberg [29]).

numbers with seizures, high blood pressure, and auto accidents. Controls had more breast tumors, cancer and anemia. None of these differences were statistically significant, and all other categories were closely similar in the two groups.

Discussion

The close similarity of exposed and control responses to questionnaires attests to the success of the matching procedures. Nevertheless the higher pregnancy rate of exposed women is still found, and the data suggest that they have higher conception rates despite similar patterns in family size, number of children wanted, and use of contraceptives and induced abortions.

If the fertility differences we have observed are related to their prenatal x-ray exposure, some mechanism of action needs to be postulated. Experimental studies have shown that low doses of radiation at sensitive stages may kill many small oocytes, but at the same time may accelerate the development of those remaining to a more mature stage (25-27). Peters has shown that fertility is not correlated with the total number of oocytes, but is correlated with the number of growing and large oocytes present in the ovary at maturity (7,28). Exposed animals may have increased numbers of large oocytes at some stages relative to controls even when the total number of oocytes is drastically reduced, as shown in figure 3 (29). Lindop tested the effects of radiation on

the reproductive performance and on oocyte numbers by stage, of mice exposed to various doses of x-ray. Although all dose levels ultimately reduced the reproductive capacity, low doses (5-10 rads in air, 10-20 rads in nitrogen) produced a temporary increase in number of litters per mouse to levels above controls early in reproductive life (27).

We have evidence that the higher fertility of exposed women is diminishing with time. Exposed rates were 23% above controls under age 16, 10% higher at 17-18, and 9% at 19-23. By calendar year, exposed rates were 15% higher than controls in 1960-1969, and 7% higher in 1970-75. In the same time periods, exposed rates of fetal deaths and spontaneous abortions increased from 18% to 33% above control rates. We speculate that x-ray exposure at some critical time in prenatal life may damage some but accelerate the development of other oocytes so that exposed girls reach puberty with more mature ovaries and oocytes, reducing the number of post-menarchal anovulatory cycles and increasing the probability of early conception. If the parallel with animal studies holds, we would expect that the excess fertility would decline with age, that fetal and neonatal losses might increase, and that the total reproductive capacity might ultimately be less in exposed than in control women. We might also expect an increase in the incidence of ovarian pathology such as cysts and tumors in these women, as well as other physical changes. These hypotheses will be tested with data now being accumulated.

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DISCUSSION

B.E. OPPENHEIM: In your 1973 paper you demonstrated that when the groups were stratified by hospital pay status, there was no difference in the fertility rates of the offspring of the welfare patients, which comprised about a third of the total group. Since the offspring of the exposed welfare patients had the same insult to their gonads as the offspring of the exposed non-welfare patients, why did they not show a similar impairment in fertility?

Mary B. MEYER: First of all, I must point out that fertility was increased rather than impaired. Our speculation on this question is that, at the lowest socio-economic levels, exposed and control women have reached the upper limits of reproductive capacity. Therefore the differences only show up at higher levels of socio-economic status.

THYROID CANCER ASSOCIATED WITH RADIATION EXPOSURE Dose-effect relationship

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Abstract

THYROID CANCER ASSOCIATED WITH RADIATION EXPOSURE: DOSE-EFFECT RELATIONSHIP.

An increased incidence of thyroid cancer has been reported in survivors of the nuclear disasters at Hiroshima and Nagasaki, in the Marshallese Islanders accidentally exposed to fallout and among children treated with medical X-ray exposure for benign conditions in the head and neck area. The latter group, having received a known exposure usually given by a standardized technique, provide an opportunity to determine fairly accurately the influence of radiation dose on the incidence of thyroid cancer. A follow-up programme currently under way directed towards over 5000 people who were irradiated between 1938 and 1955 has resulted in nearly 1200 people coming forward for examination. A significant "abnormality" was found in 288 subjects (24.2%) of whom 186 have undergone surgery so far with a finding of cancer in 61 people (5.1%). Radiation exposure dose was established for each subject using appropriate depth-dose data, Roentgen-to-rad conversion and back-scatter factors. Correlations were found between the incidences of detectable "abnormalities" and thyroid cancers with radiation dose. The projected incidence of cancer was 8.6% ($\pm 1.2\%$) at 700-750 rads and 19.4% ($\pm 6.1\%$) at 1350-1500 rads based on findings in the patients who have undergone surgery so far. The overall projected incidence of thyroid cancer in relation to dose (mean dose 783 rads, range 50-1500 rads) and period of follow-up (mean follow-up 28 years, range 19-35 years) was 3.7 cases per million at risk per rad per year. These findings are discussed in relation to the observations of other authors.

1. BACKGROUND

The possible association between radiation therapy to the neck region in young children and the subsequent development of thyroid cancer was first suggested in 1950 by Duffy and Fitzgerald [1]. Over the ensuing years many investigators confirmed the association and by the late 1950s ionizing radiation had been virtually abandoned in the treatment of infectious, inflammatory and allergic conditions in children. Some estimates of the relationship between radiation dose and cancer incidence have been made [2-4]. Renewed interest in this problem was stimulated recently by the report that a continuing incidence of cancer was observed in persons who had been irradiated more than 20 years previously and the realization that a large number of people remained at risk who had received radiation therapy to the neck region during childhood [5].

Michael Reese Hospital was one of several centres where this type of treatment had been carried out. A search of our radiation therapy records yielded the names of more than 5000 people (Table I) who had received radiation therapy to the neck region. While the majority were under ten years old at the time of irradiation, there were also some adolescents and adults. The patients were divided according to the target area, and the radiation therapy technique depended on the therapeutic objectives. Nearly

TABLE I. RADIATION-INDUCED THYROID CANCER: POPULATION AT RISK AND PATIENTS EXAMINED ACCORDING TO SITE OF THERAPY

Anatomical site	Population at risk		Patients examined
Tonsils and nasopharynx	4080	(79%)	1016
Thymus	129	(3%)	24
Other head and neck	422	(8%)	24
Other chest	333	(6%)	16
Miscellaneous	202	(4%)	112
Total	5166	(100%)	1192

80% of the patients, 4080 in all, had received X-ray therapy to the tonsils and nasopharynx using a standardized technique of parallel opposed lateral fields using 200-kV X-rays, 50-cm source skin distance, field size 8×10 cm, and HVL 1 mm of copper. For dosimetry calculations, roentgen to rad conversion factor of 0.945 and back-scatter factor of 1.33 were assumed [6].

Most of the patients received a total of three exposures of 125 R to either side of the neck at weekly intervals, resulting in a mid-plane dose calculated to be about 725 rads $\pm 10\%$. For the group as a whole the individual doses ranged from 50 rads to 1500 rads with a mean value of 790 rads, and follow-up period ranged from 19 to 35 years with a mean of 28 years.

2. METHODS

The follow-up programme was initiated in January 1974 and has been continued to the present. The primary objective was to alert patients to the treatment which they had received and the potential risks associated therewith and to invite them to undergo a diagnostic screening programme which included a careful physical examination by at least two physicians and a radionuclide image of the thyroid using ^{99m}Tc and a γ camera equipped with a pinhole collimator [7]. A palpable nodule on physical examination and/or a clearly defined area of diminished radionuclide uptake were recorded as "abnormalities" and the patients concerned were recommended to have surgery.

3. RESULTS

By March 1975, 1192 patients had been located and examined of whom 310 were found to have an "abnormality" and were recommended to consult a surgeon. Of these 199 had undergone surgery at the time of this analysis, revealing 63 cancers of the thyroid (or 5.3% of the total) and non-malignant lesions in the remainder.

From the information available on all the "abnormal" patients it did not appear that there had been selection in regard to those who had chosen to have surgery or not, as the case may be, but rather that individual patients' and physicians' opinions influenced the choice. For this reason we believe that we can expect a similar proportion of cancers and benign conditions among the remaining patients in the abnormal group. This would give a projected 98 cancers in the whole group or 8.2% of the total. Extending these projections to the population at risk, one might say that if 199 patients were operated on to reveal 63 cancers, we expect 98 cancers in the 310 abnormalities and 1192 patients examined; then we would expect a total of 425 cancers among the population at risk.

Using this projection and the mean dose and follow-up periods of 790 rads and 28 years referred to, we project an incidence of 3.7 cancers per million persons at risk per rad per year. The risk has been calculated by Silverman and Hoffman [4] on the basis of other published data, and their estimates range between 2.2 and 6.1 cancers per million at risk per rad per year.

Although it is unlikely that there is a linear function relating dose, time and frequency of cancers, there is not sufficient information at present to define the shape of the function accurately. Assuming a linear relationship with an incidence of 4 or 5 cancers per million per rad per year, with an initial latent period, the function would be as depicted in Fig.1.

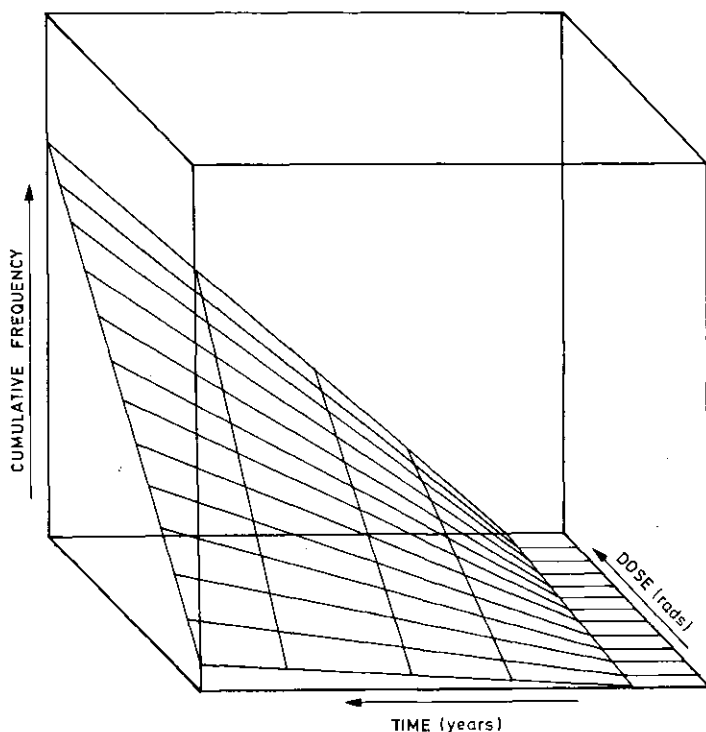


FIG.1. Radiation-induced thyroid cancer: relationship between time, dose and cumulative frequency, assuming initial latent interval and linear relationship, e.g. 4-5 cases per million per rad per year.

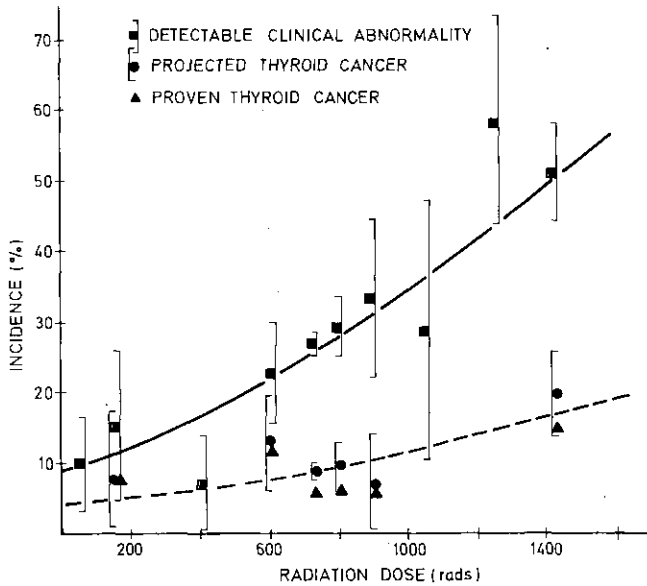


FIG. 2. Risk factors in radiation-induced thyroid cancer in relation to radiation dose.

Returning to our own data (Fig.2), we have plotted the prevalence of detectable clinical abnormality (solid line) and projected thyroid cancers (broken line) in relation to the radiation dose. At 700 rads dose, 26% of patients had a detectable clinical abnormality and 8% had cancer rising to 49% detectable clinical abnormality and 19% cancer at 1425 rads.

The study is on-going and other factors such as age at the time of irradiation, latent interval, and the continuing incidence of malignant tumours are being evaluated.

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DISCUSSION

G.W. BEEBE: The three-dimensional graph (Fig.1) contains no suggestion of a flattening or decline in the incidence of thyroid cancer over time. From the history of the leukaemia effect of atomic bomb radiation and from preliminary data on thyroid cancer in atomic bomb survivors, we would expect at least some flattening after 20 years

M. COLMAN: The three-dimensional diagram is a model if the figures arrived at by various people, namely 4 cancers/ 10^6 /rad/year, represent a linear relationship. We have not had the opportunity to observe our population of patients over a period of time. The study was started in January 1974, and the patients have been studied only once to date. Whether the figure flattens out or continues at a different slope can be determined by re-examining the patients who are normal at present over the next five, ten or fifteen years.

K. SHIMAOKA: Hempelmann and co-workers reported a high incidence of thyroid cancer among young Jewish females who were irradiated. Have you looked into the ethnic grouping of your study population?

M. COLMAN: The population which was treated at our hospital was predominantly Jewish. But this is not recorded in the charts and we have not really looked at that aspect. I am aware of Dr. Hempelmann's deductions but have not closely examined them.

W.H. ELLETT: A levelling-off or decrease in the cancer incidence may have taken place already with the highest cancer rate occurring during adolescence. Have you examined death certificates in the study population?

M. COLMAN: No, not so far.

INTERPRETATION OF NEAR-BACKGROUND ENVIRONMENTAL SURVEILLANCE DATA BY DISTRIBUTION ANALYSIS*

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Abstract

INTERPRETATION OF NEAR BACKGROUND ENVIRONMENTAL SURVEILLANCE DATA BY DISTRIBUTION ANALYSIS.

In recent years, environmental impact assessment of nuclear facilities has become synonymous with the accumulation and interpretation of near-background environmental radiation surveillance data. However, data interpretation techniques have not completed the transition from dealing with readily detectable atmospheric radioactivity contamination to the very low levels of radiation now present. Regulatory requirements for collection and interpretation of low-level environmental surveillance data necessitate the development of more effective data interpretation techniques. The application of distribution analysis techniques to interpretation of near-background data is presented as it applies to several important problem areas. Specifically treated are (1) the quantification of typical background environmental levels and expected maximum variability of these levels, (2) systemization of seasonal or yearly variations in background, (3) identification and differentiation of plant contributions to environmental contamination inventories from naturally occurring contamination, and (4) derivation of annual average concentration numbers from data including less-than-detectable values. Present methodology being applied to these problem areas is discussed and compared with capabilities and mechanics of distribution analysis techniques. The mechanics of applying distribution analysis methodology are illustrated by organizing authentic environmental surveillance data and analysing several of the example problem areas by these techniques. The objective of this discussion is to present the mechanics of application in a format for easy and direct application to problems related to the interpretation of near-background environmental surveillance data.

INTRODUCTION

Since 1963, the year that the principal nuclear powers agreed to stop open-air nuclear weapons' tests, man-made environmental radiation levels have declined considerably. However, occasional air tests of nuclear weapons by nonagreement countries continue to add to the worldwide fallout inventory. Also, expansion of the nuclear power industry presents some potential for release of radionuclides to the environment.

Current environmental radioactivity levels, although greatly reduced, are the subject of extensive measurement efforts. As the radioactivity levels in the various media of interest (air, water, soil and foodstuffs) have declined, the capability to detect the radioactivity has improved but has scarcely kept pace with the requirements. As a result, much of the data being collected in present environmental surveillance programs are valued near or less than minimum analytical detectable levels (MDL). Understanding the behavior of radionuclides released to the environment is

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essential to the systematic assessment of environmental and public health impacts. Numerous international symposia have addressed various aspects of this understanding [1-6].

PRESENT TECHNOLOGY

Environmental radiation surveillance uses the technique known as "pathway analysis" to determine points in a radiation exposure pathway at which measurements can be made and related to dose to man. A popular example of an exposure pathway is the air → grass → cow → milk → man pathway for radioiodines. Measurements may be made anywhere along this pathway but in this example are most conveniently made in the air, grass or milk.

Concepts for estimating dose to man from environmental media measurements and pathway analysis include:

The maximum "fence post" dose available to an individual present on the facility boundary.

The "maximum individual" whose dietary and recreational habits maximize the dose he might receive.

The "fifty mile population" dose potentially received by the population residing within a fifty mile radius of the facility.

Once paths have been defined, an appropriate surveillance program is established and continued to retrieve adequate data and make appropriate evaluations in terms of population impact.

CURRENT PROBLEMS

Environmental radiation is composed of natural cosmic and terrestrial radiation, global fallout radiation, and contributions from peaceful nuclear applications. Table I is presented to place in perspective the relative contributions of these sources.

As is apparent in Table I, the nuclear industry contribution to the population radiation dose in the United States is a tiny fraction of the other sources; this fraction is the subject of extensive environmental surveillance. The relatively small magnitude of the radiation dose contribution and the correspondingly small environmental radionuclide concentrations make assessment of environmental impact of these releases a formidable task.

Basic regulatory guidance on the release of radioactivity to the environment requires that such releases be as "low as is reasonably achievable" [8,9,10]. This terminology is further defined as meaning as low as is reasonably achievable taking into account the state of technology and economics of improvements in relation to benefits to the public health and safety, other societal and socioeconomic considerations, and use of atomic energy in the public interest. This guidance allows for some consideration of the cost/benefit of the environmental sampling and analysis program but also is vague enough to pressure nuclear facilities to extreme measures to 1) avoid environmental releases and 2) provide elaborate monitoring equipment and programs to detect potential releases.

TABLE I. SUMMARY OF ESTIMATES OF WHOLE-BODY ENVIRONMENTAL RADIATION DOSES TO THE UNITED STATES POPULATION [7]

Source	Annual Man-rem (millions) for Years				
	1960	1970	1980	1990	2000
Natural					
Cosmic	8.2	9.2	10.7	12.5	
External gamma	11.0	12.3	14.2	16.6	19.3
Internal	4.6	5.1	5.9	6.9	8.0
Subtotal	23.8	26.6	30.8	36.0	41.7
Fallout					
External gamma	1.1 ^(a)	0.18	0.21	0.25	0.29
Inhalation	0.27 ^(a)	0.008	0.009	0.11	0.013
Ingestion	1.0 ^(a)	0.63	0.83	1.0	1.3
Subtotal	2.4 ^(a)	0.82	1.1	1.3	1.6
Other					
Reactors	0.000016	0.00043	0.0061	0.023	0.056
Fuel reprocessing	-	0.00017	0.0050	0.025	0.065
Worldwide ³ H	0.0031	0.0092	0.0071	0.0067	0.0084
Worldwide ⁸⁵ Kr	0.00002	0.00008	0.0007	0.004	0.012
PNE tests	0.00003 ^(b)	-	-	-	-
Nevada Test Site	0.0088 ^(c)	-	-	-	-
Other AEC installations	0.0026	0.0025	0.0027	0.0033	0.0038
Subtotal	0.015	0.012	0.022	0.062	0.15
TOTAL	24.8	27.4	31.9	37.4	43.4
Population (millions)	183	205	237	277	321
Man-rem/10 ⁶ people	136 000	134 000	135 000	135 000	135 000

(a) 1963 value. A 1960 total fallout value of 1.0 was used in the TOTAL of all environmental radiation.

(b) 1962 dose; not used in totals. PNE is peaceful nuclear explosions.

(c) September 15, 1961 to September 15, 1962 dose. This value was used in the 1960 totals.

The Energy Research and Development Administration (ERDA) and the Nuclear Regulatory Commission (NRC) require that nuclear effluents and environmental effects be monitored. Facility monitoring specifications are incorporated into contractual and license agreements, and the agencies exercise surveillance inspection and independent measurement audit of the facilities. Interpretation of the collected data in terms of environmental and human impact from facility contributions is essential but difficult because of the natural variability background levels and the relatively

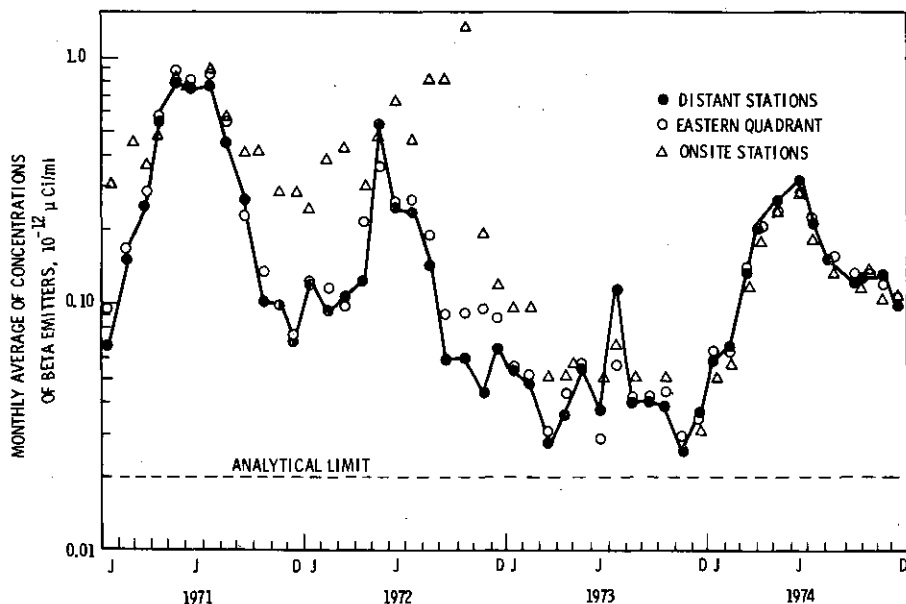


FIG. 1. Monthly average gross β activity in the atmosphere.

small increments above background which must be detected. The data obtained may be in terms of less than MDL and may be less than expected background levels due to unusual climatic, atmospheric or other cyclic or periodic factors beyond the control of the surveillance program. An example of the cyclic nature of background atmospheric beta radiation in the vicinity of a nuclear facility is shown in Figure 1.

Also shown on Figure 1 are data from predominantly downwind (eastern quadrant) monitoring stations showing possible facility contributions at some points but also showing less than background values at other points. Apart from the obvious potential facility contribution during late 1972, a consistent method is needed to quantitatively assess the less obvious potential contributions during other periods.

Distribution Analysis

Large numbers of nuclide/medium combinations exhibit log-normally distributed environmental concentrations when plotted for a location over time or for one time over many locations. That is, many environmental data groups are distributed such that they yield a straight line when plotted on log-probability paper. Although the log-normal distribution has not been applied on a wide scale to radiological data and does not seem to describe all nuclide-medium combinations, a number of authors have demonstrated its usefulness for predicting the levels of radionuclides in the environs and in human diets [11-15]. In fact, Michels [16] suggested that when the applicable distribution is unknown, a log-normal one is the first alternative to try. Because the data are presented graphically, the mean, standard deviation, expected upper limits, and any plant contribution can be readily determined visually.

Characteristics of special importance in the use of log-probability curves are linearity (denoting data from a common source), standard geometric deviation (SGD) (indicating variability in time or space), and geometric mean (\bar{X}_G). Even though the SGD of concentration curves must be determined experimentally, many sets of media background concentrations have "slope" values near two. Greater SGDs or nonlinearity usually denote the presence of a source-related concentration distribution. Quoting with confidence a geometric mean of value less than MDL is possible by means of distribution analysis and overcomes the divisive problem of how to deal with "less-than" results.

Mechanics

Mathematical data-transformation techniques have been devised for investigating characteristics of data sets. The objective of most of these transformations is to yield a straight line when the data are arranged on a cumulative probability plot. In a number of cases, special graph papers eliminate the need for an explicit transformation of the data since the complete process of data transformation is implicit in the data plotting.

The advantages of handling environmental surveillance data as distributions instead of individual data points and graphically instead of numerically are that these distribution plots:

Yield, without mathematical rigor but with the same or less effort, geometric mean values and standard geometric deviations that are the same as those obtained by numerical methods;

Quickly show whether or not the distribution choice was correct (as between Gaussian vs log normal);

Show whether or not the data belong to two statistical groups.

Groups of data including 10 to 100 items, the range of group sizes most common in environmental programs, can be handled efficiently by graphical methods. For example, weekly or monthly data may be grouped for annual reports; daily data can be conveniently summarized for monthly reporting. Sampling sites are similarly limited to less than 100 in number. These graphical methods are equally useful for larger groups of data, but manual treatment becomes tedious, particularly for routine applications.

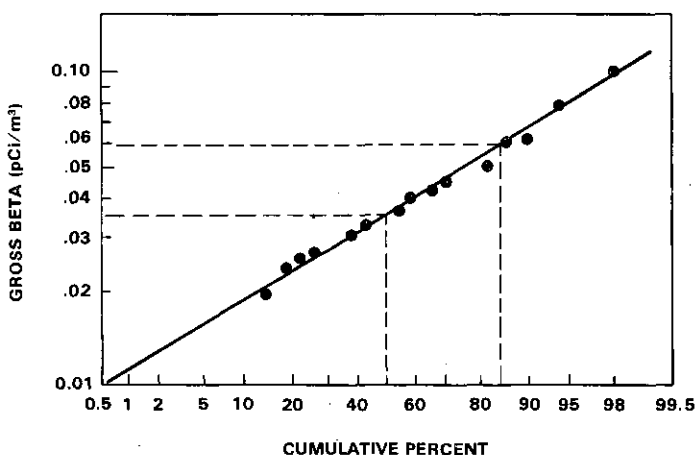
Statistical validity in plotting requires that samples be combined only into homogeneous groups. (Members of a homogeneous group can be collectively described by a single mean value and standard deviation.) These homogeneous groups may be composed of data for a single sampling location over a prolonged period of time or for a single sampling period over many sampling locations.

A set of typical data for air particulate samples collected at a given location as a function of time can demonstrate the log-normal data handling technique. The data are from biweekly samples in which the observed gross beta concentrations ranged from <0.015 to 0.1 pCi/m^3 (Table II). In this case, as in any others, the complete set of data to be analyzed is arranged chronologically (or over space) as typically entered into data record books.

To construct a cumulative frequency plot, first the raw data entries (Column I, Table II) are arranged in order from smallest to largest (ranked data). The second step in preparing the distribution plot is calculation

TABLE II. EXAMPLE GROSS BETA ACTIVITY IN AIR

Column I	Column II	Column III	Column I (Cont'd)	Column II (Cont'd)	Column III (Cont'd)
Raw Data	Ranked Data	Cumulative Percent	Raw Data	Ranked Data	Cumulative Percent
.060	<.015	2	.051	.037	54
.031	<.015	6	.043	.040	58
.040	<.020	10	.026	.043	62
.045	<.020	14	.027	.043	66
.062	.024	18	.031	.045	70
<.020	.026	22	.037	.051	74
.037	.027	26	.078	.051	78
<.015	.031	30	<.020	.051	82
.033	.031	34	.100	.060	86
<.015	.031	38	.037	.062	90
.024	.033	42	.051	.078	94
.031	.037	46	.043	.100	98
.051	.037	50			

FIG. 2. Log-normal distribution of example gross β activity in air.

of the cumulative percent for the X_i s by using the equation of Hahn and Shapiro [17]:

$$\text{cumulative percent} = 100 (i - 1/2)/n$$

The data are now ready to be plotted on log-probability graph paper.

When data are plotted on probability paper, some variation about a straight line exists because of statistical fluctuations. A best straight line drawn through the data yields values for the \bar{X}_g and SGD; the geometric mean is given by the fiftieth cumulative percent intercept, while the SGD is indicated by the "slope" of the line. Operationally, the SGD is obtained

as the ratio between values for cumulative percent intercepts which correspond to any whole unit of standard deviation (most commonly the ratio in value for intercepts of 84.1 and 50 cumulative percent).

For the example plot in Figure 2 the geometric average is 0.035 pCi/m³ and the standard deviation is 1.7. The linearity of the data as plotted in Figure 2 indicates adherence of this data group to the log-normal distribution. Experience has shown that a wide range of concentrations, shapes and SGDs are possible for similar plots of environmental surveillance data.

For interpretational reports it is desirable to use the arithmetic quantities for concentrations, etc. To do so, a derived arithmetic mean (\bar{X}_a) can be obtained from the \bar{X}_g and the SGD using the following equation:

$$\bar{X}_a = \bar{X}_g \exp \left(\frac{\ln^2 \text{SGD}}{2} \right)$$

DISTRIBUTION ANALYSIS APPLICATIONS

Typical Background Levels

An important element of log-normal data analysis is treating the data as groups, not as individuals. Consequently, the determination of data or sample representativeness changes from a task of evaluating the relationship between one entry and adjacent entries to a graphical or mathematical assessment of the entry's fit in the governing distribution. Figure 3 shows a log-normal plot of background ¹³¹I in milk. The distribution's background nature is established by two means: 1) the linearity of the plot and 2) the SGD = 2.3. Nonbackground characteristics such as plant contributions would be reflected by making the plot bimodal or by having a much greater than two SGD, depending upon the relative contributions of background and facility related sources.

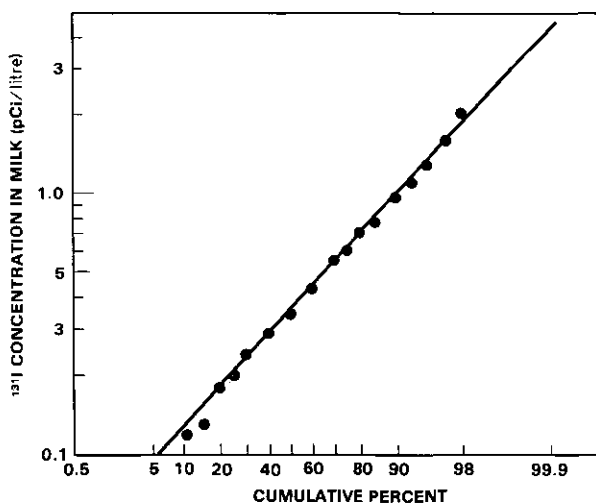


FIG. 3. Log-normal distribution of ¹³¹I in milk.

Handling of "Less-Than" Values

Because environmental monitoring programs sometimes require state-of-the-art technology applied to vanishingly small amounts of contaminants, some analyses do not detect the substance sought. For many environmental measurements, particularly those from which a chemical or instrumental background must be subtracted, net values may be obtained that are lower than the MDL of the system; individual measurements resulting in zero or negative values are not uncommon. In spite of the fact that a negative value for an environmental arithmetic concentration measurement does not represent a physical reality, a valid long-term arithmetic concentration average (e.g. annual average environmental concentration) of many measurements can be obtained only if all of the values (both the very large and the very small, including zero and negative values) are included.

If a large fraction of the data group indicates environmental concentrations below the MDL, special considerations are needed to determine the long-term average concentration and the standard deviation. A number of methods have been devised and are commonly used [14] to deal with this problem, including the assumption that 1) all less-than values are at MDL, 2) all less-than values are zeroes, or 3) all less-than values are at some value such as one-half of the MDL. Another method of averaging is to use the actual net counting data, adjusted for sample size, etc., including the smallest or negative values with the larger, positive values. The assumption that all MDL samples actually have concentrations equal to the detection limit or equal to zero can severely bias the computed average and should be avoided. The classical method of summing data and dividing by the number of data used in the sum always is algebraically correct. However, for severely skewed data, a common feature in log-normal distributions, the sum is dominated by the few largest data and thus the conventional arithmetic average is not a good measure of central tendency. Hence, the uncertainty about whether this average is close to the environmental average is dominated by the (statistical) unrepresentivity of those few largest data.

A preferred choice of handling values at or below the MDL involves probability plotting. As described earlier, the data are ranked by size, assigned percentile values and plotted on probability paper. The MDL values span the range of percentile values in accord with the fraction of the group they represent, as shown in Figure 4. The greater than MDL values are plotted in their respective percentile positions and a best straight line is fitted through them. As before, the 84.1%/50% ratio of the best straight line yields the SGD, and the geometric mean value is obtained from the intersection of the fiftieth percentile with the fitted line. This method succeeds even when more than half of the data are less than MDL since the fitted line can be extrapolated to the fiftieth percentile in any case, as done in Figure 4. When more than half the data are below the MDL, this method yields a \bar{X}_g value smaller than the MDL. The method is valid so long as a few data exceed the detection limit, although as a practical matter the confidence level may be low if fewer than 10 positive values are involved with the fitting.

In Table III the results of several averaging methods for the data presented in Figure 4 are compared. The log-normal method results in the same arithmetic average ($\bar{C} \equiv \bar{X}_a$) concentration as determined by the use of the actual net counting data, thought by some to be the only arithmetically correct method. This comparison is shown to indicate the validity of using the log-normal method. By using the log-normal plot, one

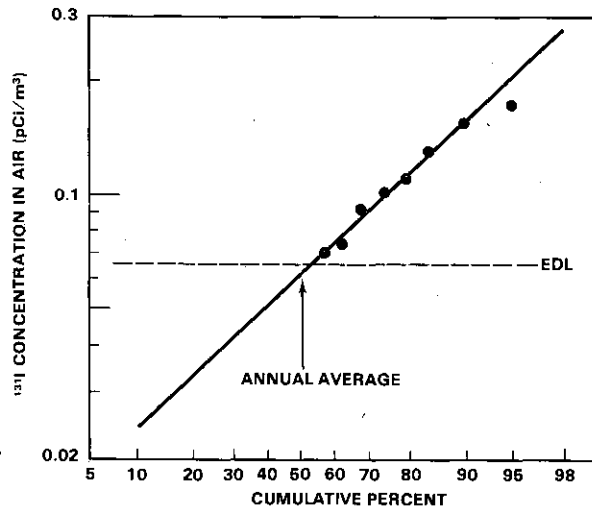


FIG. 4. Log-normal distribution of atmospheric concentration of ^{131}I .

TABLE III. CALCULATION OF ANNUAL AVERAGE CONCENTRATION

Concentration (pCi/m ³)	Cumulative Percent	Concentration (pCi/m ³)	Cumulative Percent
<0.066	5.3	0.070	57.9
<0.066	10.5	0.074	63.2
<0.066	15.8	0.093	68.4
<0.066	21.1	0.100	73.7
<0.066	26.3	0.110	78.9
<0.066	31.6	0.130	84.2
<0.066	36.8	0.154	89.5
<0.066	42.1	0.170	94.7
<0.066	47.3	0.210	100.
<0.066	52.6		

METHOD 1: Use MDL values for all less-than numbers;
quote numbers as less-than.

Result: $\bar{C} = 0.093$

METHOD 2: Use MDL values and zeroes for all less-than values and
state result as range.

Result: $0.058 \leq \bar{C} \leq 0.093$

METHOD 3: Use actual net counting data for all numbers.

Result: $\bar{C} = 0.075$

METHOD 4: Use log-normal plot to determine geometric mean
(Figure 4).

Result: $\bar{C} = 0.076$

can determine whether or not the data belong to a single distribution. If not, then an arithmetic average of the entire data set would not be valid. Thus, the only appropriate evaluation of the data would be through the use of probability plotting.

Assessing Facility Releases

After groups or subgroups of data have been arranged so they are separately homogenous, averages and standard deviations may be obtained from the probability plots. These may in turn be tested quantitatively with ordinary statistical methods. Results of these numerical tests may verify the subgrouping done on the graphical bases or will establish the differences, if any, related to geographical or chronological groupings. Clearly, some groups would represent local background conditions for the time interval of interest. Log-probability data shown in Figure 5 from Michels [16], indicate more than a single distribution mode, by jointed lines through the plotted data.

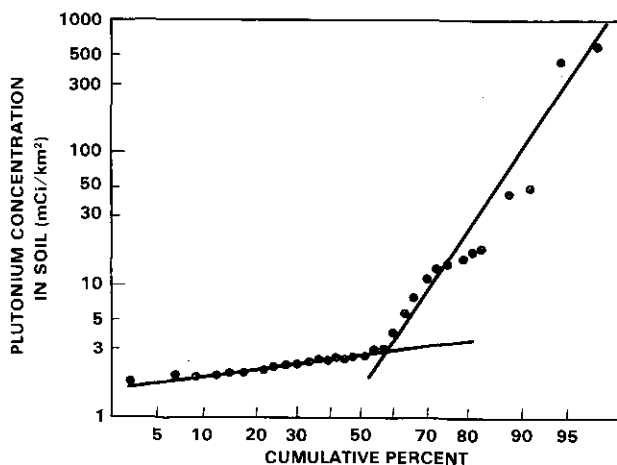


FIG. 5. Log-normal distribution of plutonium in soil.

In order to determine the extent of the background and contamination distributions, the data from Figure 5 can be reassigned assuming mixed distributions with little overlap. The lower value distribution can be interpreted as background. The presence of a distribution mode that is distinct from background indicates local contamination. The geographical limits of the contamination levels shown in Figure 5 can be mapped by referencing the sites identified by the two distributions. For all of the data shown it should be emphasized that the presence of only one "slope" (and at a relatively small value, less than about 2.5) indicates background concentrations, whereas bimodal distributions suggest an outside influence, probably from a local nuclear installation since most environmental programs are conducted in the vicinity of such installations to assess facility impacts, if any.

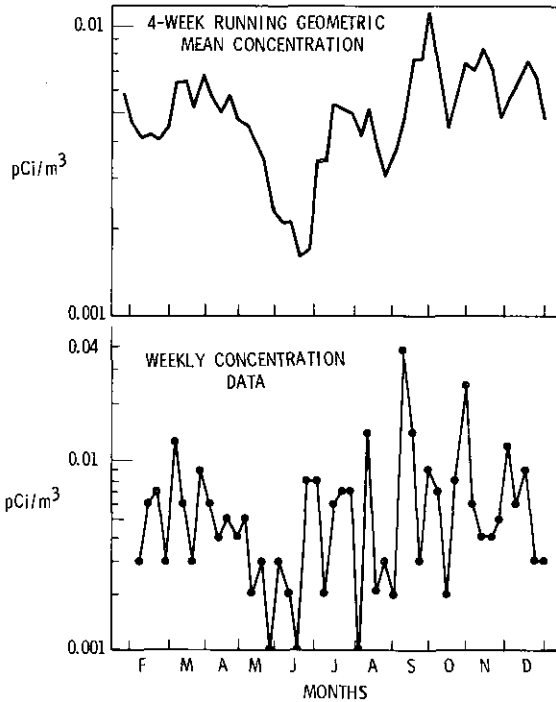


FIG. 6. Comparison of long-term indicators.

Seasonal Variation

The 84.1%/50% ratio of a log-probability curve is an index of the random statistical variability to be expected in environmental surveillance data, but systematic variations in data occur on a seasonal cycle with nuclide concentrations. An excellent way to incorporate awareness in surveillance programs for these yearly fluctuations involves plotting a running geometric mean of concentration versus time on a semilog graph.

Figure 6 illustrates the advantage of using the running mean of the data points to determine seasonal fluctuations or other long-term trends. The significance of apparent deviations from the expected value is generally determined by assessing the occurrence probability of such a deviation by conventional control chart methods. Because the purpose of the running geometric mean chart is to identify abnormal concentrations, in the interpretation one must recognize that the underlying distribution of values is log normal. Since the analytical values are distributed log normally, the proper method of finding the geometric mean is to take the n th root of the product of the n values being averaged. Four data values have been used to obtain each geometric mean which is incorporated into each point. If desirable, a warning limit based on each distribution's expected variability characteristics can be established and incorporated into this treatment.

SUMMARY

By using log-normal distribution for interpreting environmental surveillance data, assessment of local nuclear facility impacts on the environment, if any, is apparent through the change in "slope" of the log-probability graph. No change confirms that no detectable influence has occurred from local releases. The key to this kind of analysis is to treat the data as groups, not as individual data points. Because the technique suggested is graphical, whether or not the assumption of log normality is correct can be identified visually.

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DISCUSSION

F. STEINHÄUSLER: In our investigations (Steinhäusler, F., Pohl, E., Proc. 2nd European IRPA Congress, Budapest, 1973; (Steinhäusler, F., Health Phys. 29 (1975) 705) we have found that it was essential to use, in addition to the graphic plot, a quantitative test method for the goodness of fit. The transformed data can be checked for fit with a Gaussian distribution by applying the χ^2 test and the Kolmogorov-Smirnov test.

D.A. WAITE: We have found that too, and we commonly employ goodness-of-fit tests where this is warranted by the data and their ultimate use.

J. RUNDO: I should like to point out that the log-normal distribution has been applied to problems in radiological protection by various people for some years past. Quite recently we have shown that the rates of radon exhalation by persons with no known exposure to radium can be well described by a log-normal distribution. I would disagree with your use of the mean of a log-normal distribution as the "value to be expected". Surely this should be the mode of the distribution - the value which occurs most frequently.

Have you considered the use of the asymmetric normal distribution, i.e. the sum of halves of two normal distributions which have the same mean but different standard deviations?

D.A. WAITE: The point is well taken. We use the standard geometric mean, however, because it can be converted directly into the arithmetic mean, which is the value most often utilized in reporting environmental surveillance results.

We have not tried asymmetric normal distributions to describe our data, but the technique certainly seems to be worth trying.

P.G. GROER: It seems that your α counts include radon daughters. I should like to point out in this connection that varying meteorological conditions like temperature, atmospheric pressure and surface wind velocity cause tremendous fluctuations in the airborne concentrations of radon daughter products. So I would question the wisdom of using total α counts in your analysis.

D.A. WAITE: These samples were stored seven days before counting to allow for decay of radon daughters.

METHODOLOGY OF MEASUREMENT AND STATISTICAL EVALUATION OF RADIATION BURDEN TO VARIOUS POPULATION GROUPS FROM ALL INTERNAL AND EXTERNAL NATURAL SOURCES*

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Abstract

METHODOLOGY OF MEASUREMENT AND STATISTICAL EVALUATION OF RADIATION BURDEN TO VARIOUS POPULATION GROUPS FROM ALL INTERNAL AND EXTERNAL NATURAL SOURCES.

Knowledge of the radiation burden from natural sources to man in his normal environment is of great importance in our time. However, it is not sufficient to estimate only the average dose for a large population; it is desirable to know the dose to all organs and tissues with high radiation sensitivity for different groups of the population. Knowledge of such a dose distribution pattern for various population groups would enable future epidemiological studies to be carried out on possible biological effects due to low-level irradiation. The following components have to be investigated: (1) Cosmic rays. (2) External radiation from subsoil, building materials and air. These measurements should be carried out not only outdoors, but mainly in living, working and sleeping rooms. (3) Content in the air of ^{222}Rn , ^{220}Rn and their short- and long-lived decay products, both outdoors and indoors. This component causes the largest radiation burden from natural radioactivity to some tissues. These measurements are complicated by the fact that indoors and outdoors there are not only large local variations (up to a factor 300) but also temporal fluctuations at the single sites up to a factor 10. A suitable method for obtaining temporal significant means from grab-sampling is presented. (4) Radioactivity of drinking water and food. With these results the frequency distribution of the mean dose values in different organs for groups of a given population can be calculated. The statistical treatment of the data and the necessity to consider additional parameters about the population are discussed.

1. INTRODUCTION

The main obstacle to establishing maximum permissible dose levels for the general public or for certain groups of the population is insufficient knowledge of biological effects on man due to continuous low-level irradiation. Low dose rate experiments with animals are very difficult and expensive. On the other hand, extrapolation from high to low dose rates is still a problem, as is the application of results obtained from experiments with animals to man. Investigations have therefore been carried out on

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populations living in areas with an elevated radioactive environment in order to detect radiobiological effects. Since such populations often differ in their socio-ecological structure (e.g. in India or Brazil) from populations in highly industrialized countries, differences in possible biomedical parameters detected might not be caused by ionizing radiation alone.

This paper should show that significant differences of the natural radiation burden can be found within every country, even within a small region such as a town. It is possible to group the population of a region according to the radioactive environment in which it is living. However, attention must be paid to the natural radiation background that can cause a very different dose for various tissues and organs. Knowledge of the mean "dose distribution pattern" in the organism for the member of a single group would make it possible to carry out future epidemiological studies on biological effects.

Numerous investigations have been undertaken on one or another component of the natural background. However, it is absolutely essential to gain, at least, a detailed knowledge of all important components of the natural sources of ionizing radiation. External and internal irradiation show large local and temporal variations due to many influencing factors. This variability requires extensive measurement programmes at sites, selected according to defined criteria. Due to the complexity of the task, a suitable methodology of measurement is necessary. It must comprise the following:

- (a) Consideration of all factors causing variations of the natural radioactivity; and
- (b) Compromise between the vast amount of radioactivity data required and the practicability of sampling and measuring methods.

Additional information about the life pattern of the population under consideration and application of selected statistical methods on these data will reveal significant values, which are necessary to group the population for the dose assessments.

The radioactive environment of a highly urbanized area will differ a great deal from that of an agricultural area. Large differences exist in the accumulation of construction material (houses, streets), increased use of coal and/or natural gas (all acting as sources of natural radioactivity) as well as in the time spent indoors and outdoors.

2. SOURCES OF NATURAL IONIZING RADIATION

2.1. Cosmic rays

Numerous investigations have been carried out on cosmic rays and their variation with elevation, geomagnetic latitude, barometric pressure and solar cycle. The cosmic rays in the lower atmosphere consist of an ionizing and a neutron component. Unfortunately, the data available are still very inconsistent. Despite the large amount of data on the ionizing component, discrepancies up to 40% between the results reported can be found in the literature [1-3]. According to the review of several measurements, the most probable value for cosmic ray ionization density at sea level is $2.44 \text{ ions} \cdot \text{cm}^{-3} \cdot \text{s}^{-1}$ at NTP, which is equivalent to 35.3 mrem/a [4].

Even fewer results, but with very large variations, are available on the neutron component [5-7]. In the present study 3.5 mrem/a are suggested at sea level at 40° latitude for the average tissue-absorbed dose rate [8]. To calculate the dose from cosmic rays for various altitudes we suggest the use of data in Ref.[4].

The influence of the barometric pressure and the solar cycle is only small and can therefore be neglected, in view of the other uncertainties mentioned. The dependence of the ionizing component on the geomagnetic latitude amounts to about 10-15%, whereas no precise data are available for the variation of the neutron component [9].

Further investigations are needed for the alteration of cosmic rays inside multistorey buildings (shielding effect), where a large part of the population in our civilization spends its time.

2.2. External γ radiation

The major sources from the external environmental natural γ radiation are the nuclides: ^{208}Tl , ^{214}Bi , ^{228}Ac , ^{214}Pb , ^{212}Pb and ^{40}K . The concentration in the ground of the members of the ^{238}U series, ^{232}Th series and of ^{40}K accounts for the largest amount of the γ dose received outdoors. Under normal atmospheric conditions a relatively small contribution to the γ flux of about 2-3% results from the radon daughters contained in the air [10], while, due to enrichment in inversion layers, it can be increased up to 20% [11]. In agricultural areas, these natural radionuclides contained in phosphate fertilizers are an additional source. From occupational external exposure a dose rate of 2 mrad/a has been estimated for agricultural workers [12]. In towns we find major alterations of the external γ radiation compared with rural areas outdoors: the γ radiation is enhanced due to the nuclides contained in construction material of houses and roads, while the component from the subsoil is reduced.

Indoors, the γ dose level is determined mainly by the content of ^{226}Ra , ^{232}Th and ^{40}K in the building material used. The material acts as an additional source of radiation as well as shielding material against the γ radiation outdoors. The γ radiation from the radon daughters must also be considered.

At present, very different kinds of building material are in use. Recent extensive investigations of building materials [9,13] show that the mean radioactivity of the different groups differ remarkably, but samples from within the same group can also reveal large variations. It is therefore always necessary to study the specific local conditions.

Temporal variations of the external γ radiation at the same site have been found outdoors and indoors. The reasons are: change in the soil moisture in the top 5-10 cm of the soil, therefore influencing soil density and radon diffusion into the atmosphere; wash-out of radon daughters from the atmosphere to the soil [14]; varying concentration of radon daughters in the air indoors.

2.3. Inhalation of ^{222}Rn , ^{220}Rn and their progenies

A very important, but often not investigated, component of natural radioactivity is represented by ^{222}Rn , ^{220}Rn and their short-lived daughter products. Due to exhalation from the soil and from the construction material

of buildings, they are always present in our atmosphere outdoors and indoors. These nuclides are incorporated into the human body by inhalation. In parts of the respiratory system this causes the highest radiation burden from natural sources.

The radon concentration outdoors varies a great deal locally and temporally, depending on the concentration of ^{226}Ra and ^{232}Th in the soil and on meteorological parameters. The latter influence the rate of exhalation and the stability conditions in the atmosphere. The concentration of the daughter products outdoors depends mainly on the existing vertical exchange and advection of air masses in the lower layers of the atmosphere [15].

Indoors, radon concentration shows up as large local and temporal variations due to the influence of several factors. Locally dependent factors are: the ^{226}Ra and ^{232}Th content of the building material, the location of the building and the location of the room inside the building. The following factors are time dependent: exhalation from the surrounding walls and from the soil underneath, ventilation conditions, and the radon concentration outdoors.

The two factors, building material and ventilation conditions, account for most of the variations found. The rate of radon exhalation from the building material is largely influenced by the finish of the surfaces (paints, wall-paper, panelling, etc.).

The concentrations of the short-lived daughter products indoors are predominantly determined by the rate of ventilation. In inhabited rooms radioactive equilibrium is hardly ever reached. The time variation of radon and daughters indoors is very large and is also influenced by several meteorological parameters [15].

2.4. Natural radionuclides in the diet

An important natural radionuclide in food is ^{40}K . Since potassium is part of the organic substance, a constant level in the single tissues is reached due to the metabolism, independent of the ^{40}K content of the diet. Sex and age dependence has been investigated extensively [9].

Other contributing natural radionuclides are ^{226}Ra , ^{238}Th , ^{210}Pb and ^{210}Po , with an average concentration of about 1 pCi/kg [9]. Drinking-water contains on the average about 10-100 $\mu\text{Ci/litre}$ of ^{222}Rn and short-lived decay products and about 0.1 pCi/litre of ^{226}Ra [9]. The amount of these nuclides in food or water is determined by their (or by their mother nuclide's) concentration in the subsoil at the production site of the food or the area of the wells, and therefore large deviations from the above values are possible. As for the natural radionuclide content in fertilizers, the amount of uptake of these nuclides by growing plants needs to be investigated further, especially considering the concentration effect in the plant-animal-man food chain found in areas with an elevated level of natural radioactivity [9]. Under normal living conditions the contributions to the dose from these nuclides in food and drinking-water are very small.

3. METHODOLOGY OF MEASUREMENT

As an example of such a study, we present our investigations in Salzburg, Austria, started in 1974. Some of the preliminary results are presented in order to demonstrate such a programme.

The city of Salzburg (150 000 inhabitants) is situated 420 m above sea-level on the northern slope of the Alps. As is typical for a large number of European towns, the inhabited houses comprise constructions from mediaeval up to recent days. The building material used originates from areas with very different geological structure.

3.1. Pre-operational planning

At the beginning of the investigation the area of the town is divided into smaller districts. Usually, such a subdivision already exists at the local municipal department of statistics. It is recommended to keep to the same division into districts because then all additional statistical data can readily be used from that department. The number of measurements in each district is then chosen according to the number of people living in the district. Important information can be obtained from the data on the frequency of certain professions, age and sex within a single district. From these data can be assessed approximately how much time is spent indoors and outdoors. In general in our civilization most people spend the largest part of their time (up to 90%) indoors, especially in northern countries. Therefore it is absolutely essential to carry out far more measurements indoors than in the open air.

3.2. Cosmic rays

Measurement of cosmic rays would require very special dose meters with linear response up to very high energies. In most cases it is sufficient to calculate the dose for the site from data published in the literature, taking geomagnetic latitude and elevation into consideration.

3.3. External γ radiation

For field measurements, a light-weight portable instrument is needed, with isotropic and (up to 2.6 MeV) energy-independent response. A large time constant or the use of a recorder is necessary. We use the low-level scintillation dose meter developed from Kolb and Lauterbach [16] with highest sensitivity of $3 \mu\text{R/h}$ full scale. To determine the part of cosmic rays, which is detected simultaneously with the terrestrial component, measurements have to be carried out on a large enough lake. A possible variation with altitude has to be taken into consideration; however, for the above instrument we found only a small dependence on heights up to 1000 m. For a given height, all γ measurements are reduced by the dose rate determined in this way.

In the defined districts γ measurements are undertaken outdoors in front of the selected site and indoors. Since it can be rather difficult at times to obtain the owner's permission to perform measurements in their flats, we often take the reading in the basement and on the staircase at several floors. These measurements were found to agree satisfactorily with those taken from within the flats. All readings are taken at about 90 cm above the ground as a representative height for gonad position. Some authors use TLD dose meters exposed at selected sites indoors. However, temporal variations cannot be detected and the possible effect of surface contamination from radon daughters needs further investigation.

For further statistical evaluations the whole set of data from all measurements is then tested for frequency distribution. Sometimes the data will show very skewed frequency distributions and suitable transformations have to be found. Goodness-of-fit of a Gaussian distribution with the transformed values can be checked by applying the χ^2 and Kolmogorov-Smirnov test [17].

As an example for the variations within a relatively small area (0.7 km²), the extreme values of all outdoor measurements in Salzburg cover the wide range 15-131 mrad/a. A summary of recent results indoors in comparison with the preliminary results of our measurements is contained in Table I. The mean dose rates differ up to a factor of 2 but, what is even more important, the extreme values cover the wide range from 1 mrad/a to over 300 mrad/a. This emphasizes the need for determination of dose frequency distributions.

TABLE I. EXAMPLES OF RECENT MEASUREMENTS OF TERRESTRIAL GAMMA DOSE RATE IN BUILDINGS (WITHOUT COSMIC RAYS)

		No. of measurements	Gamma dose rate (mrad/a)		
			Mean	Min.	Max.
Ohlsen [18]	Fed. Rep. Germany	667	74	1	314
Yeates and King [19]	Australia	200	70	-	-
Lindeken et al. [20]	USA	~100	41 ^a	30 ^a	108 ^a
Kiefer et al. [21]	Fed. Rep. Germany	~2500	64	11	285
Present authors	Austria	1480	48	16	156

^a Corrected against the component from cosmic rays by the present authors.

3.4. Radon and decay products

The following information is required for dose calculations from these nuclides: the concentrations of ²²²Rn, ²²⁰Rn and their short-lived decay products; the percentage of decay products unattached to atmospheric aerosols; the size distribution of atmospheric aerosols.

To assess mean concentration values for a given site, we have developed a sampling method which improves the calculation of mean values from grab sampling. At a few sites, which are selected on the basis of the construction material and with defined ventilation conditions, "control stations" are installed. At these stations daily long-term measurements of ²²²Rn, ²²⁰Rn, ²¹⁴Pb and ²¹²Pb are carried out with double-filter devices [22]. In addition, ²²²Rn samples are collected in working, living and sleeping rooms, using specially developed air bags. These samples are measured with an ionization chamber connected to a high-input resistance FET electrometer [23]. To correct against diurnal fluctuations, the deviation of the ²²²Rn concentration at the different control stations from the long-term mean is used as a factor of correction. Applying these factors for every-day

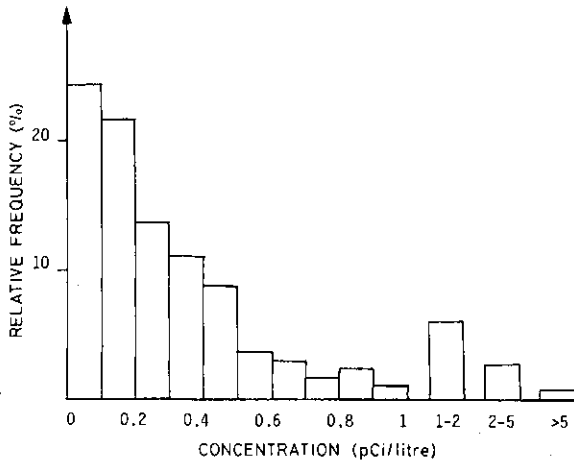


FIG. 1. Relative frequency histogram in percentage for annual mean radon concentration indoors.

grab sampling, all those corrected ^{222}Rn concentration values represent temporal mean values for the respective sites. Furthermore, radon samples are taken repeatedly at every site at different seasons to obtain radon values for varying ventilation conditions. As an example, Fig.1 represents our preliminary results of the frequency distribution for the corrected ^{222}Rn data. Since the correction against temporal fluctuations has been applied, even the extreme values represent annual mean values.

The uncombined fractions and the particle size distribution of the daughters can be determined simultaneously with the nuclide concentrations. Suitable methods using fine wire screens or diffusion batteries have been developed [24].

3.5. Diet

From statistical data on food consumption by the population under investigation, typical meals are defined for the daily main courses. Food samples from different brands are then collected for these meals and measured for their content of ^{226}Ra , ^{228}Th , ^{210}Pb and ^{210}Po with suitable low-level spectrometer systems.

Data are available from the local waterworks about the origin of the drinking-water supply for the different districts of the town. Samples for determination of ^{222}Rn and ^{226}Ra are taken at the consumer's mains. For further details about water sampling and method of measurement see Ref.[23].

4. DOSE CALCULATIONS

For the dose calculations it is necessary to determine the life pattern of the population, mainly the time spent indoors and outdoors in different buildings (home, working place, etc.). From the whole set of measure-

ments, statistically significant mean values for the radioactive environment are calculated for every population group. These data are then used to assess the doses for the single organs, since the term whole-body dose is meaningless for all other components of the natural radioactive environment except cosmic rays.

4.1. External irradiation

For calculating the tissue-absorbed doses to different organs it is necessary to know the shielding factors by the body for each organ depending on age and sex. However, the few data available are still inconsistent.

4.2. Internal irradiation

The rare gases ^{222}Rn and ^{220}Rn inhaled are transported with the bloodstream and distributed almost uniformly in most tissues. Although the daughter products are primarily deposited in the respiratory system, part of them is also transported with the blood to all other organs; however, their distribution is very non-uniform. For extensive experimental investigations and dose calculations see Ref.[25]. Dose assessments for the lung, and especially for the bronchial epithelium, are very inconsistent. For detailed discussion of the various models see Ref.[9]. Such calculations are difficult for children, since no applicable age-dependent lung model exists. Assessments, however, reveal much larger doses for children.

TABLE II. DOSE RANGE OF SALZBURG POPULATION FROM NATURAL RADIOACTIVITY FOR DIFFERENT ORGANS

Sources of radiation	Ranges of dose rate (mrem/a)			
	Gonads	Bone marrow	Alveolar tissue	Bronchial epithelium (4 th - 9 th generation)
<u>External:</u>				
Cosmic rays	55	55	55	-
γ rays from soil, building material and air	15 - 150	5 - 60	5 - 60	-
<u>Internal:</u>				
^{40}K , ^{14}C , ^{226}Ra ^a	23	17	~20	-
^{222}Rn b, decay products, ^{212}Pb	0.2 - 18	0.3 - 37	30 - 4700	230 - 37500
Total	93 - 246	77 - 169	110 - 4835	230 - 37500

^a Calculated from data in the literature.

^b Minimum: women, light work, living and working rooms with 0.1 pCi/litre radon
Maximum: men, heavy work, living and working in rooms with 8 pCi/litre radon
Dose calculations for the respiratory system [26].

Little information is available on the radiation burden due to inhalation of ^{210}Pb . The inhaled ^{210}Po causes a radiation load mainly to the bronchials. However, the dose is only a small portion of that from the short-lived decay products [9]. For calculations of the dose from the ^{40}K content of the body and from the natural radionuclides in the diet and drinking-water see Ref.[9].

5. CONCLUSION

The methodology of dose assessment from the natural radioactive environment must account for the complex superposition of the many components contributing to the dose as well as for the large temporal and local variability of the data.

The individual dose received from external irradiation is determined mainly by the location of the home and the place of work and by the building material used. Variations in dose of a factor 5 are likely. The dose from internal irradiation depends on the mean radon and daughter-product concentration which can vary locally up to a factor 300. Age, sex, profession and spare-time occupation determine the respiratorial minute volume with differences of the extremes up to a factor 10. This in turn reflects the amount of radon daughters inhaled.

All this makes it desirable to determine the frequency distribution of the dose received by the different groups of the population, subdivided for several organs. Such calculations are very elaborate and require a large amount of data. Our investigations in Salzburg have so far revealed the dose range for the population (Table II). Considering that, even within as small a region as a town, the ratio of annual mean doses is up to 1:160 for the respiratory system and 1:2.5 for other organs, it seems very necessary to reconsider the use of the term "mean whole-body dose".

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DISCUSSION

R.B. HOLTZMAN: During your presentation you stated that the ^{226}Ra level in the water was about 35 pCi/litre, which is very high in comparison with the values found in Illinois waters around Chicago. How much of this water is actually ingested in cooking and in other beverages consumed in the region of study?

F. STEINHÄUSLER: The drinking water is supplied from several wells situated in different areas. The water from these sources is collected and mixed in large reservoirs, which supply all the needs of the consumer for cooking and so on.

R.B. HOLTZMAN: ^{210}Pb may contribute a substantial fraction of the skeletal dose, especially in high radon areas.

F. STEINHÄUSLER: I should like to add that the actual measurements differed by a factor of up to 5 for the dose from external irradiation and by a factor of up to 300 for the mean concentration of radon and its daughters. Depending on the life pattern, age and sex, the resulting total dose differs by a factor of up to 160 for the respiratory system and by a factor of 2.5 for other organs. These differences cannot be said to denote the existence of uniformity.

R.B. HOLTZMAN: Factors of 3 in environmental measurements are not really extreme and may indicate fairly uniform conditions.

H. WIJCKER: Is the water treated for hardness by the drinking-water utilities? If so, what is the reduction factor for Ra, which belongs to the same group as Ca in the periodic table? Do you think that there may be a change in future with increasing knowledge of the beneficial influence of hardness on heart and vascular diseases in man?

F. STEINHÄUSLER: The utilities supplying water to the public do not use softeners. Such devices are used, however, in some private homes and apartment houses. In our measurements, we could not detect any significant reduction of the ^{226}Ra concentration in drinking water due to treatment with softeners.

At present, we have no information about any plans for a change in water treatment.

M. GOLDMAN: In view of the local extremes in spatial and temporal dose estimates, would it not be appropriate also to add biomedical factors

such as influence of nose versus mouth breathing on "lung dose" estimates from α emitters? Do you ascribe any health consequence to these large variations in small doses?

F. STEINHÄUSLER: Yes, I am sure that these factors play an important role. However, we feel that this is mainly a concern of the Lung Task Group established by ICRP and should be considered as part of a refinement of lung-model studies.

Regarding your second question, future epidemiological studies on population groups whose radioactive environment has been assessed in the manner described could well reveal biomedical differences in effects due to low-level irradiation.

CHROMOSOME ABERRATIONS IN PERIPHERAL BLOOD LYMPHOCYTES DEPENDENT ON VARIOUS DOSE LEVELS OF NATURAL RADIOACTIVITY*

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Abstract

CHROMOSOME ABERRATIONS IN PERIPHERAL BLOOD LYMPHOCYTES DEPENDENT ON VARIOUS DOSE LEVELS OF NATURAL RADIOACTIVITY.

The additional α blood dose, due to inhalation of air containing an elevated amount of ^{222}Rn , to the population in the area of the 600-year-old radon spa Badgastein (Austria) encompasses mean values from 2 mrad/a for groups of residents, up to 1500 mrad/a for workers in a special treatment centre, located in a former gold mine. The external dose caused by terrestrial sources, subsoil, building materials and radon decay products, and by cosmic rays (sea-level 800 - 1250 m) ranges from 80 to 300 mrad/a. Chromosomal aberrations in the peripheral blood lymphocytes have been investigated in 122 persons, with 30590 cells scored. The dose rates, accumulated in the last six months before sampling, were estimated individually for every single person, taking into account the various times spent at different locations, the respiratorial minute volume during their activities, and the significant mean values for the radon and decay product concentrations in the air at these locations. For people who received an ($\alpha+\gamma$) dose < 100 mrad and an α dose < 5 mrad the age dependence for each kind of aberration was calculated. For all other cases the aberrations have been corrected for age and their dose dependence was then analysed. A non-linear relationship was found with an initial sharp increase up to 200 mrad, followed by a flattening of the curve for chromosome aberrations, whereas the chromatid aberrations showed no dose dependence.

1. INTRODUCTION

Many studies have been carried out on radiation-induced chromosome aberrations. However, only limited information exists on chromosome aberrations due to continuous low-level irradiation, and still less is known of the influence of low-level α irradiation. Such investigations are only possible on a population exposed to radiation exceeding the normal background.

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We have studied the inhabitants of the spa Badgastein, situated in the Austrian Central Alps, whose radioactive environment consists mainly of elevated concentrations of ^{222}Rn and its daughters in the atmosphere. This increased air activity is produced by 19 thermal springs emerging in the centre of the town. Five million litres of hot water pour out daily and contain a total amount of about 200 mCi ^{222}Rn . This water is conducted from the springs to big reservoirs, and from there to 130 hotels and spa houses, where it is used for treatment. Almost all of the radon emanates into the air. In addition, an excess of radon is released from the subsoil in the whole region because of water-conducting fissures and veins, and also because of the relatively high radioactivity of the geological subsoil. Moreover, the latter contributes to the γ radiation as well as to the air activity since it provides part of the construction material for the local buildings.

The air activity is highest in rooms with treatment facilities, and persons living near such rooms or working in them (e.g. bath attendants) receive higher radiation doses than the other inhabitants. According to the outdoor and indoor air activities, we have defined two zones in Badgastein. Zone I encompasses the area where the thermal springs originate, and Zone II consists of all the other peripheral regions. Treatment is also carried out in a former gold mine near the town, called the "Thermal Gallery". In this gallery there are air temperatures of up to 41°C, a relative humidity of about 95%, and a mean radon concentration of 3 nCi/litre. As the short-lived decay products reach 70 - 90% of the equilibrium value, this corresponds to about 25 WL. The gallery, a giant hot radon inhalation room with 22 000 m³ of air, is at present used for the treatment of rheumatic and vascular diseases as well as metabolic and endocrine disorders. A large spa establishment has been built in front of the gallery, which houses the medical facilities, resting and waiting rooms, etc. This building is directly connected to the entrance of the gallery, which results in an elevated radon concentration of the air in the whole house, with mean values of between 8 and 150 pCi/litre for the various rooms. The decay products yield from 0.1 to 0.8 WL. Patients are of no interest to this investigation, as they receive only small doses during their ten two-hour sessions in the gallery. However, there are groups of persons receiving considerably larger doses. The largest dose is received by the miners who inspect the gallery and the traindrivers who bring the patients into the mine, followed by that of the doctors accompanying the patients. They stay in the gallery 2 - 4 hours daily for several months per year. Another group with smaller radiation doses consists of the attendants and other personnel working and living in the spa house for 60 hours a week. A special group is the caretaker and his family living the whole year in the house. The latter receive their elevated radiation dose almost continuously, whereas the others get their dose fractionated as they live and sleep in houses with lower activities.

2. DOSE ASSESSMENTS

In our previous analysis the population of the whole region was divided into seven groups with various radiation dose levels, and we used as a control group inhabitants of the surrounding areas, who live on the same geological substratum and have the same socio-ecological conditions but

with lower radon content in the atmosphere [1 - 3]. Table I gives a survey of the environmental radioactivity at the various locations. These data are the result of a thorough survey of all the components of natural radioactivity in the whole region derived from thousands of measurements with ionization chambers and filter devices (for ^{222}Rn , ^{220}Rn and decay products), as well as scintillation and LiF-glass dose meters for the γ irradiation [4, 5].

The external γ dose varies, for all the persons under consideration, between 90 and 250 mrad/a. As the special radioactive environment of the Gastein area results from the elevated ^{222}Rn content in the atmosphere, the main radiation load is due to internal irradiation resulting from the inhalation of ^{222}Rn and its short-lived daughters. The contributions of the long-lived progenies as well as of ^{220}Rn and its daughters are only small. From the significant mean values for the air activity, we calculated the doses due to inhalation that are different for the various organs and tissues. These calculations were based on results from our previous investigations on the dose distribution pattern after radon inhalation [6].

For this final and detailed analysis of the experimental data it was necessary to calculate the doses accumulated over a certain period separately for each person investigated and to consider not only the differences in the α blood dose, but also in the γ blood dose due to external γ irradiation from terrestrial and cosmic origin as well as from diagnostic X rays. However, these differences do not vary by more than a factor of 3 for the individuals. As the time period for dose accumulation, we chose six months before the day of blood sampling. For calculating the dose, information was collected from each person individually on the amount of time spent at the working place, at home, in the open air or elsewhere. Air activity and γ dose measurements were carried out at all these locations. In addition, the kind of activity was taken into consideration for calculating the α blood dose. This is very important, as differences of up to a factor of 10 exist between the respiratorial minute volume during hard manual work and while sleeping. After collecting all these data for each person investigated, we calculated the α blood dose as follows:

$$D_{\alpha} = 0.015 \sum \text{Rn}_i t_i + 0.156 \sum \text{Rn}_i t_i z_i \frac{\Phi_i}{\Phi_N} \quad (1)$$

D_{α} : α blood dose in mrad for half a year

Rn_i : radon content in pCi/litre at site (significant mean values)

t_i : time in hours spent at site (from personal information)

z_i : factor calculated from decay-product ratio (according to measurements):

if $\text{Rn}:\text{RaA}:\text{RaB}:\text{RaC} = 1:a:b:c$

$$z = \frac{795 a + 4640 b + 1706 c}{7141}$$

$\frac{\Phi_i}{\Phi_N}$: ratio of actual to standard respiratorial minute volume corresponding to activity:

	<u>sleeping</u>	<u>light activity</u>	<u>walking</u>	<u>heavy work</u>
Man:	0.52	2.0	3.0	4.3
Woman:	0.35	1.2	1.5	1.8

TABLE I. MEAN AIR ACTIVITIES AND TOTAL EXTERNAL RADIATION IN THE AREA OF BADGASTEIN

	Mean air activities (pCi/litre)					Total external radiation (mrad/a)
	^{222}Rn	^{214}Pb	^{210}Pb	^{220}Rn	^{210}Pb	
<u>Badgastein</u>						
Zone II: open air	0.8	-	-	0.5	0.0014	80 - 150
Zone II: room air	2 - 5	1 - 3	-	0.3	0.007	100 - 190
Zone I: open air	2	1	Up to 0.01×10^{-3}	0.6	0.002	80 - 170
Zone I: room air	5 - 11	3 - 8	Up to 0.17×10^{-3}	0.4	0.008	120 - 300
Rooms connected to thermal bath-rooms	45	18	-	-	-	200
Thermal bath-rooms	90	30	-	15	0.015	300
<u>Mine region</u>						
Treatment house at entrance to thermal gallery	30	15	Up to 0.53×10^{-3}	-	-	250
Thermal gallery	3000	2400	310×10^{-3}	8 - 50	1 - 6	1500

The γ dose was calculated as follows:

$$D_{\gamma} = \left[(D_h t_h + D_w t_w + D_b t_b) \times 0.66 + D_{out} t_{out} \times 0.78 \right] \times 1.1 + D_{cr} \times \sum_{i=1}^n t_i \quad (2)$$

D_{γ} : γ dose for half a year

D_h, D_w, D_{cr} : dose rate at home, at work and due to cosmic rays

D_b, D_{out} : mean dose rate in buildings and outdoors of home village

t_h, t_w, t_b, t_{out} : time spent at home, work, other buildings and outdoors

The factor 1.1 in Eq.(2) converts the measured dose rate (R/h) into tissue-absorbed dose rate (rad/h), and the absorption factors for the organ blood for indoor and outdoor exposure are 0.66 and 0.78 respectively. These latter we estimated to be slightly higher than the mean values for all organs published by UNSCEAR [7]. The cosmic ray dose was interpolated according to the height above sea-level of the various living areas [7].

3. CYTOGENETIC INVESTIGATIONS

The blood sampling was taken from the cubital vein. From the higher dose groups, samples were taken repeatedly up to six times, at intervals of half a year to four years. Routine blood culture techniques were employed, and where possible early harvests were scored. All deviations from the normal were noted, i.e. aneuploid and polyploid cells and all chromatid and chromosome aberrations. These included gaps, chromatid breaks and exchanges, fragments, minutes, dicentrics, and morphologically abnormal chromosomes. Two or more events in one single cell were additionally scored as multiple aberrations. No ring chromosomes were seen.

Only chromosome aberrations of the so-called unstable type were included in the estimation of break rate. Deletions other than those accompanying multicentric chromosomes were scored as one-break events; minutes and dicentric chromosomes as two-break events. As isochromatid breaks or gaps of NuPD type cannot with certainty be distinguished from terminal deletions, aberrations of this type, although scored separately, were counted as fragments in assessing the breakage rate. 122 persons were investigated, and a total of 30 590 cells scored.

4. DATA ANALYSIS AND RESULTS

A preliminary analysis of the mean α blood doses and associated aberration rates for each of the seven groups has been published [1 - 3]. This showed no difference within the first four groups, followed by an increase in groups six and seven. The upturn point seemed to be between 30 and 100 mrad/a. A later analysis with more data showed an age dependence for some of the aberrations. Our material normalized to an age of 50 years showed the same dose dependence.

With the above results as a basis, we combined all cases whose accumulated ($\alpha + \gamma$) doses were less than 100 mrad in half a year, whereby the α dose had to be less than 5 mrad. These cases (56 of the total of 177) were investigated for age dependence. Computer analysis of 56 cases tested against age dependence showed no significance for aneuploidy and

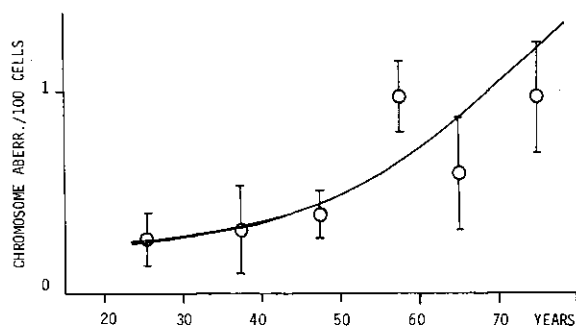


FIG.1. Age dependence of chromosome aberrations.

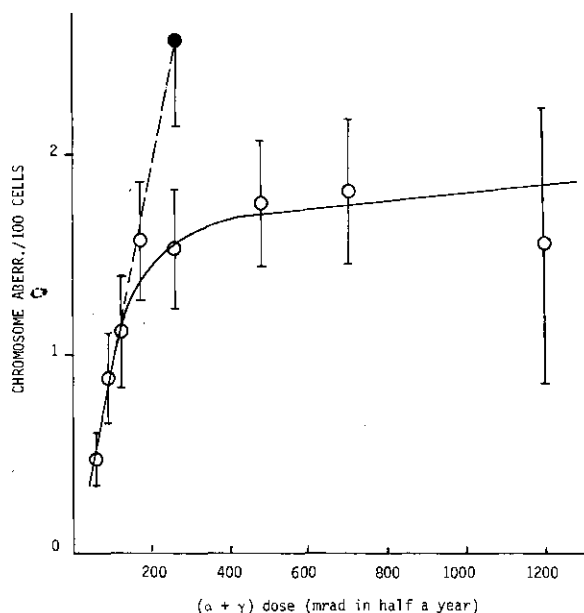


FIG.2. Dose dependence of chromosome aberrations.

polyploidy. A linear dependence for chromatid gaps and breaks with a weak statistical significance $P < 0.05$ was present. For fragments, dicentrics plus minutes, and morphologically abnormal chromosomes, as well as for cells with more than one aberration, the age dependence seemed to be non-linear. This relationship is summarized in Fig.1.

The remaining 121 cases, corrected to 50 years according to the plots for age dependence, were tested against the combined ($\alpha + \gamma$) doses. This showed no dose dependence for aneuploidy, polyploidy and chromatid gaps. A weak dependence for chromatid breaks was suggested but this was not statistically significant. For fragments, for the two-break events and for the multiple events we found each a non-linear dose dependence with an initial sharp increase up to 200 mrad followed by a flattening of the curves up to 1300 mrad (our maximum dose). These results are summarized in Fig.2.

5. DISCUSSION

The present results of our investigation into the long-term effects of low-level irradiation on the chromosomes of peripheral blood lymphocytes confirm our previous findings: the existence of a significant increase in chromosomal aberrations starting at levels above 100 mrad for a period of half a year. In the present analysis, the dependence was calculated against a combined ($\alpha + \gamma$) dose. The proportion of these two components varies, however, over the dose range investigated. At the lower levels (up to 200 mrad), the γ fraction exceeds the α by a factor of up to 100, whereas at the higher dose rates the α fraction exceeds the γ by a factor of up to 10. Although it is not possible to separate the effects of these two components, it is conceivable that the shape of these curves (an initial rise followed by a flattening out at the higher levels) could be the result of different RBE of these two types of irradiation for chromosome aberrations in the peripheral blood lymphocytes.

However, a further explanation is possible. Persons investigated received their dose in different ways. Those with burdens of up to 200 mrad were irradiated continuously from the environment, whereas the additional radiation received by those with the higher burdens was a result of a fractionated dose from occupational exposure while working in the Thermal Gallery for periods of two to four hours daily. Repair mechanisms could therefore be more effective in the latter group although the actual radiation received was both greater and of higher LET.

This hypothesis is supported by the findings in the four members of the caretaker's family who live and work in the spa house attached to the Thermal Gallery. Although receiving the relatively low radiation dose of 200 - 300 mrad, but continuously, they have aberration rates higher than any other person (black point in Fig.2). One can see from Fig.2 that the extrapolation of the first increase meets this point. The dotted line is probably representative for continuous irradiation.

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DISCUSSION

M.L. GRIEM (Co-chairman): Have you analysed the chromosomes in the lymphocytes by Giemsa banding techniques as described by Janet Rowley, or by Hoechst fluorescent staining as described recently by Sam Latt (of Harvard) and S. Wolf (of the University of California, San Francisco)?

Johanna POHL-RÜLING: We have not used banding techniques but are planning to do so in the future.

W. SCHUETTMANN: In your study, did you investigate only chromosome aberrations, or did you combine this investigation with that of, for example, other haematological or biochemical parameters? If so, what are your results?

Johanna POHL-RÜLING: We performed only chromosome investigations. Another group, headed by Dr. Paletta of the University of Graz, is carrying out experiments to look for corticosteroids in rats inhaling ^{222}Rn . They have found an increase for the inhaling rats.

A.L. BROOKS: You recorded your dose in terms of that accumulated over a period of half a year. Since there is evidence for a long life in the case of some lymphocytes, it may be appropriate to consider cumulative dose over a longer time interval.

Johanna POHL-RÜLING: We were aware of what you say about lymphocytes' lifetime, but chose the short time interval because this approach suited us. However, we intend in future to consider cumulative dose over longer times.

R.B. HOLTZMAN: What is the source of irradiation of the cells studied?

Johanna POHL-RÜLING: The irradiation came from two sources:
(1) The external γ irradiation was due to natural terrestrial γ rays and cosmic rays. The blood dose which occurred there was calculated by Eq. (2).
(2) The internal α irradiation originates from the inhalation of naturally occurring ^{222}Rn and its daughters. This internal α dose is very different for the various organs. It is highest for the respiratory tract, where the airborne solid decay products are filtered off during inhalation. From here they are transported by the bloodstream to all the other organs, where they accumulate according to their metabolism. The dose distribution pattern after inhalation of ^{222}Rn and its daughters was studied in previous publications (e.g. Ref. [6] of our paper). The blood dose was calculated according to Eq. (1).

INVESTIGATIONS ON HUMAN POPULATIONS RESIDING IN HIGH BACKGROUND-RADIATION AREAS OF KERALA AND ADJOINING REGIONS

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Abstract

INVESTIGATIONS ON HUMAN POPULATIONS RESIDING IN HIGH BACKGROUND-RADIATION AREAS OF KERALA AND ADJOINING REGIONS.

Investigations on human populations residing in monazite-bearing high-background radiation areas of Kerala and adjoining regions, reported earlier, are brought up to date. The object of the investigation is to evaluate long-term effects of chronic radiation exposure in man. Demographic data, including fertility history, of 13355 households (~ 70 000 individuals) in a ~ 55-km long strip had been collected. A radiation dosimetric survey of nearly 20% of this population, using calcium fluoride thermoluminescent dose meters, had been completed. Chromosomal analyses on 35 cord blood samples from new-borns of parents resident in high-background radiation areas as well as areas with normal background radiation levels have been completed so far. The data as such did not indicate any significant differences in chromosomal abnormalities such as chromosome/chromatid breaks, acentric fragments and chromosome/chromatid gaps between samples from high and low radiation areas. Dicentric, rings, etc., have not been observed in any of these samples. Confirmation of these preliminary results would have to await the results of extensive studies now in progress.

1. INTRODUCTION

Deposits of radioactive minerals occur in littoral formations along the coastal regions of India. Of particular interest is a stretch about 250 km long and about 0.5 km wide on the SW coast in the states of Kerala and Tamil Nadu. The radioactive component of these deposits is monazite which contains principally thorium together with several rare earths; the monazite deposits are admixed with ilmenite, rutile, sillimanite and zircon along the coastal areas. The most concentrated deposits along the Kerala coast are located on a 55-km strip extending from Thekkumbhagum in Quilon District in the south to Purakkadu in Alleppey district in the north. The thorium content of the monazite here ranges from 8% to 10.5% and is the highest in the world. Additional features of this area are that it has definable geographical landmarks with the backwaters separating this strip from the mainland and that it supports very dense human and plant populations. Further south, another coastal strip about 2.5 km long near Manavalakurichi in Tamil Nadu also includes similar high concentration of monazite.

Figure 1 is a schematic representation of the decay chain of thorium and its daughter products. It is evident that during the decay of thorium, α , β and γ radiations are released — as is also radioactive thoron, which diffuses out of the sands and contributes to the contamination of the air.

Trombay, has undertaken a phased programme of investigations aimed at obtaining this information for man as well as lower biological forms. The present paper brings our programme up to date. Details of the first part of the programme have been published in a previous report [2] as well as in the open literature, and the relevant references are indicated in the text.

2. RADIOMETRIC AND DEMOGRAPHIC SURVEYS

The first phase of the programme was an assessment of the amount of environmental radiation prevalent in the monazite belt. After a preliminary examination of radiation levels at locations on the beaches with a GM counter probe, 200 houses selected at random in the 55-km strip of the Kerala coast described earlier were covered in an initial survey using an ionization-chamber mR meter [3]. Measurements of γ and $(\beta + \gamma)$ radiation level were made at the main entrance of the houses and in the rooms and courtyards, if any. The average γ radiation level showed wide variations between houses and demonstrated the rather patchy distribution of monazite along the coastal strip. On the basis of these measurements, it was observed that the average γ field in the region was about 1300 mR/a. It was also computed that the total β and γ radiation level, corresponding to the average γ level of 1300 mR/a, was 1500 mR/a.

A detailed demographic survey of the human population resident on the 55-km strip extending from Thekkumbhagum to Purakkadu was carried out with the assistance of the Bureau of Economics and Statistics, Kerala State [2,4]. The region under study was divided into seven areas and each area was subdivided into segments, and each house in a segment was given an individual house number. In all, 13 355 households are included in the region and nearly 70 000 persons, representing all age groups and different religions, are covered. A batch of investigators visited each household and the demographic data were collected in computer-compatible formats [5]. The population has been divided into three occupational groups representing: (I) individuals employed outside the area; (II) individuals totally employed within the area; and (III) fishermen. Occupational Group I includes children going to school and other individuals who spend a good part of the day outside the area, but does not include fishermen. Occupational Group II includes male members who normally work in the area, housewives, and children who do not go to school but spend the day in and around the house. Occupational Group III consists of all persons who go fishing and spend the rest of their time in and around the area. This classification is important since human beings are mobile and the fact that they are resident in a high background area may not necessarily imply that they always receive high exposures. For each married couple in a household a detailed fertility history has also been obtained.

A detailed report of the preliminary demographic and radiometric surveys and an analysis of the data may be found in the Proceedings of the 4th International Conference on the Peaceful Uses of Atomic Energy, Geneva, 1971 [4]. (See also Refs [6-9].)

It is very likely that the various demographic parameters which have been analysed are not sensitive enough to reveal any differences which are statistically significant. In view of our earlier cytogenetic findings on plant populations from high background radiation areas of Kerala and adjoining regions [10-13] and of the recent observations of radiation damage to

chromosomes of human cells irradiated in vitro, it is hoped that human chromosomal studies of the population resident in the area would throw more light on the effects of chronic high background radiation on man. Detailed studies have been planned on the blood lymphocytes from the new-borns, children of various age groups and adults, on samples drawn from the various radiation exposure groups. For this purpose a field laboratory has been set up at Chavara, close to the monazite belt.

Chromosomal analysis on 35 cord blood samples from new-borns of parents resident in high background radiation areas as well as areas with normal background radiation levels have been completed so far. The data as such did not indicate any significant differences in chromosomal abnormalities such as chromosome/chromatid breaks, gaps, etc., between samples from high and low radiation areas. Dicentrics, rings, etc., have not been observed in any of these samples. Confirmation of these preliminary results would have to await the results of extensive studies now in progress.

3. ASSESSMENT OF DIETARY INTAKE OF RADIOACTIVITY BY POPULATION

Almost simultaneously with the demographic survey of the population in the monazite belt, a detailed dietary survey covering 460 families (3210 individuals) living in 23 villages/locations along the coastal strip was undertaken [14]. Using the interview and weighing methods, the food consumption pattern of the population was determined. The complete range of food materials representing the average diet of the population was assayed for radioactivity [14]. On the basis of these data, the daily per capita dietary intake of gross α , ^{228}Ra and ^{40}K activity has been estimated to be 215, 162 and 3551 pCi, respectively. The critical foods, i.e. the foods which make the greatest contribution to the contamination of the total diet, for this population are fish and tapioca. It is felt that the present estimates of the likely intake of radioactivity would serve adequately to assess any biological risks associated with such intake levels.

Studies of Rajewsky et al. [15] and Penna Franca [16] have suggested that the placental level could serve as an index of the body burden of radium nuclides. Work has been initiated to assay ^{228}Ra levels in placenta of new-borns in the population whose blood lymphocytes are being screened for chromosomal abnormalities as indicated earlier. Analysis of a few placental samples for ^{228}Ra through radioassay of its daughter nuclide ^{228}Ac , after separating actinium with lanthanum carrier [17] and using a low-background GM counter, has been completed. Data have not revealed any detectable ^{228}Ra activity above the reagent and detector background. Further work on placenta as well as teeth and bone samples collected from residents of the monazite belt is in progress.

ACKNOWLEDGEMENTS

It is a pleasure to acknowledge the assistance of Health Physics Division, Bhabha Atomic Research Centre, Trombay, in the dosimetric surveys and of Dr. G. Kshirsagar in radiochemical assay of placental samples. The work has been supported in part by the World Health Organization.

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DISCUSSION

H.H. VOGEL, Jr.: Are the subjects of your experiment being followed for possible cancer induction at later times, and have cancer registries been established in Southern India?

Even though your results are mainly negative for the criteria investigated, these persons who have received radiation doses 10-20 times the background level should be followed in connection with long-term studies on possible radiation carcinogenesis.

K.P. GEORGE: We are looking into the incidence of cancer, especially in people exposed to high radiation exposure levels.

RISK ESTIMATES
(Session IX)

Co-Chairmen:

H. H. ROSSI (United States of America)

A. L. BROOKS (United States of America)

L'ENVIRONNEMENT DES CENTRALES NUCLEAIRES

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Abstract-Résumé

THE ENVIRONMENT OF NUCLEAR POWER STATIONS.

The author traces the evolution of the ideas of radiation protection specialists, especially in the United States of America, where the situation suddenly deteriorated in 1969. On the basis of examples, he makes a comparison between the health hazards observed in the French population and theoretical health hazards. The latter, which have nothing in common with observations, are nevertheless the subject of very sophisticated preventive measures in the environment of nuclear power stations. On the other hand, there are a number of pathological cases which, owing to the lack of adequate funds, are still too much neglected. To correct this theoretical pathology, the consequences of which would be socially disastrous, there is a need for a pilot and for a rational study of the situation, which is today a very perilous one. In conclusion, the author makes a number of constructive proposals.

L'ENVIRONNEMENT DES CENTRALES NUCLEAIRES.

L'auteur retrace l'évolution des idées des spécialistes de radioprotection, particulièrement aux Etats-Unis d'Amérique, où la situation s'est dégradée brusquement en 1969. Il met ensuite en parallèle, sur quelques exemples, des risques sanitaires observés dans la population française et des risques sanitaires théoriques; ces derniers, qui n'ont rien de commun avec les observations, font cependant l'objet de mesures préventives très poussées dans l'environnement des centrales nucléaires; par contre, on observe de nombreux cas pathologiques qui, eux, ne sont encore que trop négligés, faute de crédits suffisants. Pour libérer l'industrie nucléaire de cette pathologie théorique, socialement désastreuse, il faudrait un pilote et une étude rationnelle de la situation, aujourd'hui fort compromise. L'auteur termine sur des propositions constructives.

1. INTRODUCTION

L'environnement des centrales nucléaires est parsemé — du moins dans l'esprit de tout le monde — de cas pathologiques redoutés. La présentation de cet environnement tend à y enfoncer ces centrales, au lieu de les en libérer. Il est vrai que toute centrale nucléaire produit, et rejette, des radio-éléments qui constituent autant de sources de rayonnements ionisants. Sur ces sources, leurs idées évoluant dans le sens d'une prudence accrue, les spécialistes de radioprotection ont élaboré une pathologie toute théorique, mal perçue par les intéressés. Brusquement la situation s'est révélée mauvaise. Voyons comment nous y sommes parvenus, pour chercher à en sortir.

2. LES EVENEMENTS ET L'EVOLUTION DES IDEES

C'est essentiellement aux Etats-Unis que l'on retrouve les événements qui ont été à l'origine de l'évolution des idées des spécialistes de radioprotection. On peut distinguer plusieurs étapes dans cette évolution.

2.1. Avant la seconde guerre mondiale

Au commencement, ne sachant rien, les professionnels ont, progressivement, mis au point des méthodes de mesure des rayonnements ionisants, des règles et des moyens de protection. Tout était relativement simple. Cependant ils ont commis quelques erreurs, redoutables. Alors, ils se sont groupés pour en discuter, d'abord en Grande-Bretagne, puis à l'occasion des congrès internationaux de radiologie. C'est ainsi qu'ils ont créé en 1928 l'organisation qui devait devenir la Commission internationale de radioprotection (CIRP). Cela a conduit les diverses sociétés américaines de radiologie à se concerter pour créer ce qui devait devenir le Conseil national pour la radioprotection et les mesures (NCRP) [1].

Tous deux, NCRP et CIRP, admettaient l'existence d'une dose de tolérance qui, située nettement au-dessous d'un seuil d'effet, ne devait provoquer aucune lésion [2, p.61]. En 1934, le NCRP et, quelques mois plus tard, la CIRP, ont recommandé [1], respectivement : 0,1 et 0,2 rem/d.¹ Un effet somatique bien connu des radiothérapeutes leur avait servi de repère : l'érythème cutané; administrée en quelques minutes (non étalée sur une journée de travail) la dose susceptible de provoquer une rougeur fugace de la peau est numériquement plusieurs milliers de fois plus forte que la dose de tolérance [3, p.60].

2.2. Durant la seconde guerre mondiale

Tout est devenu plus complexe lorsque, aux Etats-Unis, on a construit des réacteurs nucléaires où des neutrons engendraient de très nombreux produits de fission, radioactifs, et des éléments transuraniens, tout nouveaux; l'un de ces derniers, le plutonium 239, devait même servir d'explosif militaire. En se multipliant, les applications de l'énergie nucléaire, militaires et pacifiques, pouvaient, si l'on n'y prenait garde, augmenter considérablement l'irradiation de l'humanité. Ainsi, dans le plus grand secret, tandis que des physiciens mettaient au point les premières bombes atomiques, d'autres chercheurs développaient leurs connaissances en radiopathologie, et sur la dosimétrie des divers rayonnements issus de sources situées à l'extérieur ou, aussi, à l'intérieur de l'organisme [4]. Si une irradiation prolongée de 0,1 à 0,5 rem/d ne semblait pas provoquer de lésion somatique, on a cru déceler de "faibles effets génétiques" pour une dose cumulée de 100 rems seulement [1, p.22] : la dose de tolérance ou, plus précisément, la garantie de sécurité, se trouvait mise en doute par l'expérimentation.

2.3. Dans l'après-guerre : principes fondamentaux

Dès 1946, réorganisé et étoffé par des chercheurs et des hygiénistes, physiciens et biologistes, le NCRP a introduit l'expression "dose admissible" [2, p.61], devenue, par la suite, dose maximale admissible (DMA). En 1951 [5], à peine reconstituée et réorganisée, la CIRP a adopté une position semblable. La DMA, substituée à la dose de tolérance, en différait bien peu numériquement (1 rem/semaine au lieu de 0,2 rem/d), mais elle en diffère fondamentalement par sa définition : la personne qui la reçoit courrait un risque, mais un risque facilement acceptable eu égard aux autres dangers de la vie. Corrélativement, après avoir indiqué que ses jugements reposent sur une connaissance insuffisante de la radiopathologie, et que certains effets des rayonnements sont irréversibles, donc cumulatifs, la CIRP [5]

¹ Pour simplifier, nous adoptons d'emblée le rem pour unité de dose. Lire rem par jour.

a vivement recommandé de réduire l'irradiation des personnes au plus bas niveau possible.

Il faut bien reconnaître que, dans la presse médicale, de nombreuses publications ne pouvaient qu'inciter à la prudence. Par exemple, les radiologues auraient subi une augmentation considérable du risque de leucémie [6], une diminution de longévité [7], une atteinte génétique de leur progéniture [8]. Pire, selon d'autres auteurs, ils rendraient des enfants cancéreux en les irradiant, soit dans le sein maternel, pour radiodiagnostic [9,10], soit après la naissance, pour radiothérapie fonctionnelle [11].

2.4. Hypothèse prévisionnelle quantitative

Aussi, en 1955, sur une Terre toute contaminée par des explosions nucléaires expérimentales à l'air libre, et sachant que la première conférence internationale sur "l'atome pour la paix" allait se réunir à Genève, deux rapports préliminaires ont été établis d'urgence [1, p.47] : le premier, à la demande du premier ministre de Sa Majesté, par le Conseil de la recherche médicale britannique (MRC) [12] ; le second par le Conseil national de la recherche de l'Académie des sciences américaine (NAS-NRC), sous deux formes, l'une relativement détaillée [13], l'autre, très résumée, destinée au public [14]. Dans ces deux rapports les plus grands spécialistes du moment ont souligné, exemples à l'appui, la gravité des dommages (somatiques tardifs et génétiques) qui pouvaient résulter de l'augmentation de l'irradiation des personnes : aucun dommage ne lui étant spécifique, cette irradiation supplémentaire alourdirait la charge de maux et de douleurs de l'humanité. Pour prévoir par le calcul quelle pourrait être l'augmentation de cette charge, en vue de la limiter de façon jugée acceptable, on a admis, pour chacun des dommages possibles, que le risque individuel serait proportionnel à la dose cumulée. De tels risques, théoriques [15], sont rapportés à l'unité de dose, soit par an, soit pour la durée de la vie; un groupe de travail de la CIRP [16] a collationné des valeurs trouvées dans la littérature.

2.5. Résultats de calculs prévisionnels

Si l'on admet la proportionnalité d'un risque (théorique) à la dose, on montre facilement [17] que le dommage collectif théorique (DCT) correspondant est proportionnel à la somme des doses individuelles cumulées dans la population considérée; appelée dose collective, cette somme s'exprime en personnes-rem en cas d'irradiation globale, ou, pour un organe particulier, en personnes - (nom de l'organe) - rem. Chacun a pu se livrer à des calculs de DCT. A titre indicatif, voici des résultats récents dus à la Commission à l'énergie atomique américaine (AEC) [18, pp. 7-63 et 5 B-19], laquelle a emprunté les valeurs des risques au fameux "rapport BEIR" de l'Académie des sciences [19]. Pour un million de personnes-rem, ou de personnes-thyroïde-rem, le calcul donne des DCT de : 26 leucémies, 110 cancers thyroïdiens, et 75 autres cancers; et pour un million de personnes-gonades-rem, à l'équilibre génétique : de 6 à 600 anomalies morphologiques, de 30 à 300 maladies dues à un caractère héréditaire dominant, et "une montée très lente" des maladies dues à un caractère héréditaire récessif.

2.6. A partir de 1969, aux Etats-Unis, puis partout

Aux Etats-Unis, jusqu'en 1969 les applications industrielles de l'énergie nucléaire paraissaient promises à un rapide développement [20] : les centrales commandées totalisaient plus de 70 000 MW, dont près de 60 000

pour 1967 et 1968. Cependant [21], le grand bruit fait par les mass media sur des accidents survenus dans des centres de recherche nucléaire, et la divulgation d'un rapport établi en 1957 par l'AEC [22], ont jeté le trouble, semé l'inquiétude, dans bien des esprits. Selon ce rapport, un réacteur de puissance moyenne (500 MW thermiques) pourrait provoquer accidentellement jusqu'à 3 400 morts et 43 000 blessures, ou justifier le paiement de 7 milliards de dollars d'indemnisations; un tel rapport devait servir de base de discussion pour établir une convention d'assurance; cependant les assureurs, secondés au besoin par les fonds publics, n'ont admis de couvrir, après de longues discussions, que jusqu'à 560 millions de dollars [23].

Et de grands savants, de notoriété mondiale, comme Albert Einstein et Robert Oppenheimer, déclaraient que l'humanité pourrait courir à sa perte en mésusant de l'énergie nucléaire; d'autres, comme Linus Pauling ou David Lilienthal mettaient nettement en garde les responsables contre les dangers de cette nouvelle forme d'énergie.

A partir de 1969, quelques personnes ont, en s'opposant au développement des applications de l'énergie nucléaire, contribué à accroître sensiblement l'inquiétude générale, et ainsi acquis un grand renom. Parmi les plus connues on peut citer un juriste (Ralph Nader) et trois scientifiques (Ernest Sternglass, John Gofman et son élève, Arthur Tamplin). Le premier fait encore frémir les foules en parlant des dangers des rayonnements ionisants ("forme nouvelle et silencieuse de violence") et de l'incurie des responsables [24]. Les autres ont simplement repris, et interprété, mais avec éclat, et excès [25 - 27], le contenu de publications parues dans la presse médicale, ou celui de textes de la CIRP et du NCRP. Gofman et Tamplin demandaient avec insistance que la limite d'irradiation des personnes du public recommandée par ces commissions, et adoptée par la réglementation, soit réduite au dixième de sa valeur. En 1971, l'AEC est devenue, brusquement, encore plus exigeante que ces opposants [28], du moins en ce qui concerne les effluents des centrales nucléaires. Peine perdue : aussitôt leur attention se portait ailleurs, et ils enfonçaient de nouvelles portes. Leur habileté, les reculades de l'AEC, rendaient vraisemblables leurs déclarations sur l'insuffisance des connaissances et sur la nécessité d'un moratoire nucléaire.

Et le monde entier observait, suivait, suit toujours.

3. NOTRE SITUATION ACTUELLE

Dans une population en croissance (en nombre et en besoins) les hydrocarbures pouvant manquer, les responsables français de l'approvisionnement du pays en énergie ont, en 1973, brusquement décidé de recourir massivement à l'énergie nucléaire. Cette décision a surpris ceux que les centrales nucléaires, écrasées de précautions, inquiètent. L'inquiétude se généralisant, eux-mêmes inquiets, ces responsables infligent à ces centrales des précautions supplémentaires pour, surtout, que la radioactivité ne porte pas atteinte à la santé des personnes. Cette santé préoccupe chacun au premier chef, et on la protège socialement.

3.1. Politique sanitaire

A l'instar des économistes, en vue d'utiliser au mieux les crédits dont ils disposent, les responsables de la protection sanitaire font procéder à des études de "rationalisation des choix budgétaires" [29]. Cependant, chacun sachant bien que "la santé n'a pas de prix", sous la pression de l'opinion, ils ne peuvent pas ne pas favoriser l'action curative, au détriment de l'action préventive et de la recherche. Et cette pression favorise certaines thérapeutiques, celle du cancer au premier chef.

La radioprotection mise à part, pour juger des effets de toute action entreprise, ils suivent l'évolution des statistiques sanitaires dans leur pays, les comparent à d'autres. Ce faisant, ils ne peuvent formuler de conclusion qu'a posteriori, et avec toute l'incertitude inhérente à l'imperfection des moyens d'observation et d'interprétation [30, 31].

En radioprotection, se donnant des risques, ils s'efforcent de prévoir quelles pourraient être, au pire, les conséquences lointaines de l'irradiation de personnes. Les calculs leur donnent des valeurs de DCT qui, quoique impressionnantes (voir ci-dessus, §2.5), ne sauraient transparaître sur les statistiques sanitaires, nous allons le voir (§4.1).

Deux traitements aussi différents ne peuvent pas ne pas engendrer des aberrations manifestes, trop souvent négligées. Pour faciliter le rapprochement de situations différentes, nous allons exprimer tous les risques en millionnièmes, le plus souvent par an; pour alléger l'expression, lorsque le contexte le permettra l'unité ne sera pas rappelée. Voyons, successivement, quelques risques relatifs à la santé publique, à la santé des travailleurs, au cas particulier des centrales nucléaires.

3.2. Quelques risques relatifs à la santé publique

Dans la population, le risque moyen de mourir d'insuffisance rénale chronique, très faible, serait voisin de 50. Des organismes publics et privés ont mis en place d'importants moyens de traitement dans de nombreux pays, dont la France, où, cependant, on ne peut encore mettre en traitement, chaque année, que 1 500 des 2 500 cas nouveaux [32]. Ce traitement réduit le risque de mort de 1 million (mort imminente) à 50 000; pour fixer les idées, rappelons qu'il est en moyenne de 10 000 environ pour l'ensemble des Français [33]. On pourrait sans doute arriver à traiter au mieux tous ces Français en 1980 en investissant encore un milliard de francs par an jusqu'à cette date [34].

Pour ceux qui, chaque année, font l'objet d'une déclaration de grossesse, les risques de mort périnatale ou de handicap à la naissance seraient, respectivement, de 25 000 et de 50 000; parmi les handicapés, on compte 8 000 débiles mentaux profonds et 7 000 cas de malformation majeure [35]. Beaucoup de ces morts ou handicaps pourraient être évités; la Suède en donne le meilleur exemple. Mais la prévention d'une mort ou d'un handicap nécessiterait la mise en œuvre de moyens supplémentaires, d'un coût très variable suivant le cas, pouvant aller de 200 F pour la réanimation en salle d'accouchement, à 100 000 F pour la prévention d'une affection, qui cause une débilité mentale profonde; même à ce prix, le dépistage systématique et la prévention de cette affection, extrêmement rare (risque voisin de 100), serait moins coûteux que le placement d'un idiot en milieu spécialisé; et cet enfant, ainsi, ne plongerait pas sa famille, désemparée, dans le malheur.

Tout ne va pas non plus pour le mieux en ce qui concerne la préservation des eaux contre la pollution : la construction d'une station d'épuration des eaux usées, d'autant plus lourde à supporter que l'agglomération est plus petite, coûterait une centaine de francs par habitant pour une petite ville. L'opinion publique n'est pas disposée à accepter de telles charges budgétaires [36]. Il est vrai que si cette pollution fait des victimes, il est bien difficile de les lui attribuer.

3.3. Quelques risques dus au travail

Le risque de mourir de cancer des fosses nasales, voisin de 10 dans une population non exposée, est augmenté sensiblement par l'inhalation de tanin : il atteindrait 130 pour certains travailleurs du cuir et 700 pour certains travailleurs sur bois [37]. Nos médecins du travail le savent bien [38, 39], mais ils savent aussi que leur imposer une prévention efficace rui-

nerait de trop nombreux petits artisans. Et ils n'ignorent pas que ce cancer n'est pas pris en charge comme maladie professionnelle : notre législation ne le reconnaît pas encore.

Un médecin du travail [40] rapporte qu'une source de 15 Ci d'iridium 192 a été retrouvée dans la poche de la veste de travail d'un étranger. Il l'avait prise pour une goupille. Semblable méprise, non exceptionnelle, peut avoir des conséquences redoutables; on en a publié des exemples [41, 42].

3.4. Cas particulier des centrales nucléaires

Si de toutes petites sources de radioéléments ont trop discrètement déjà fait de bien trop nombreuses victimes (le radium 226 arrive au premier rang) [43], l'attention est focalisée sur les centrales nucléaires. Tandis qu'elle prescrivait aux exploitants de réduire l'irradiation des personnes du public par ces centrales [28] (voir ci-dessus, § 2.6), l'AEC lançait une vaste enquête sur le niveau de la radioactivité dans leur environnement, sur l'amélioration des procédés de rétention des radionucléides gazeux et liquides, sur le coût de ces améliorations, sur la traduction de la radioactivité du milieu en doses engagées et en DCT [18]. Le tableau I montre que, sauf pour le corps thyroïde, les exploitants respectaient largement les limites dosimétriques recommandées par la CIRP; il montre aussi que les centrales récentes irradieront beaucoup moins encore.

TABLEAU I. LIMITES D'IRRADIATION (mrem/an)
Personnes du public - Réacteur à eau sous pression

Limites pour	organisme entier	peau	corps thyroïde
normes CIRP	500	3 000	3 000
anciennes	50	55	2 500
centrales récentes	0,4	0,5	8

Cependant, pour passer d'une centrale de type ancien à une centrale de type récent, c'est-à-dire pour augmenter l'efficacité des dispositifs de rétention de la radioactivité, la majoration des dépenses annuelles est évaluée à environ 5 millions de francs pour l'équipement et l'exploitation de deux tranches de 1 200 MW [18, pp. 3-1 à 3-25 et 44, pp. 116 à 131]. Et ces centrales doivent, en outre, depuis peu, résister aux séismes et à la chute d'un gros avion commercial. Or, leurs responsables évaluèrent que le risque d'une telle chute sur un stade de la banlieue de New York, alors qu'il est plein de monde, est sans doute compris entre 0,1 et 1 par an [23]; mais les organisateurs de rencontres sportives l'ignorent, et ne s'en soucient pas.

Quand on songe à tous les cas qui, faute de crédits, attendent en vain une action sanitaire préventive, ou salvatrice, on ne peut pas ne pas souhaiter que soient adoptées, concurremment pour tous les secteurs, les limites d'une protection raisonnable, y compris en radioprotection.

4. LES LIMITES D'UNE RADIOPROTECTION RAISONNABLE

Les calculs prévisionnels reposent sur des valeurs de risques d'effets tardifs obtenus dans des conditions d'irradiation très particulières. Ces valeurs diffèrent grandement de celles que fournit l'observation prolongée d'individus, ou de générations, après une irradiation, soit accidentelle, soit systématique, soit expérimentale. Voici quelques exemples.

4.1. Leucémogénèse

La moelle osseuse est sans doute le plus vulnérable des tissus de l'organisme à l'irradiation. Deux publications sont fondamentales en ce qui concerne la leucémogénèse. L'une, de Court-Brown et Doll [45], classique, porte sur l'observation d'un groupe de rhumatisants après radiothérapie localisée au rachis. L'autre, de Jablon et Kato [46], rapporte les données les plus récentes sur les causes de mort parmi les survivants des explosions nucléaires d'Hiroshima et de Nagasaki; nous limiterons ici l'observation à Nagasaki; le cas d'Hiroshima, où une fraction notable de la dose a été due à des neutrons, d'interprétation difficile, sera abordé séparément, ici-même, dans une autre communication [47].

Pour répartir leur groupe en classes, Court-Brown et Doll ont considéré, pour chaque personne, la dose dans la moelle osseuse rachidienne, Jablon et Kato, la dose dans l'air, à l'emplacement de la personne. Pour comparer les résultats, nous avons, pour chaque classe, évalué la dose moyenne dans l'ensemble de la moelle osseuse (les cinq douzièmes de la dose rachidienne, les deux tiers de la dose dans l'air), puis dressé le tableau II et dessiné les graphes de la figure 1.

En radiothérapie les doses moyennes ont été élevées, ou très élevées (700 rems dans la dernière classe, non rapportée ici). Ayant évalué le risque spontané, 50, d'après les statistiques sanitaires britanniques de l'époque, bien conscients de l'insuffisance de leurs données, Court-Brown et Doll ont émis une hypothèse de travail de recherche selon laquelle l'irradiation ajouterait un risque annuel de 1 à 1,5 millionième de leucémie par rem cumulé dans la moelle osseuse (voir les deux droites en tirets sur la figure 1). La

TABLEAU II. RISQUES ANNUELS DE MORT PAR LEUCEMIE OBSERVES APRES RADIOTHERAPIE RACHIDIENNE (Radiot.) ET A NAGASAKI (Nag.) (Doses médullaires pas très élevées)

Dose moyenne dans la moelle (rems)		Effectif (10 ³ personnes-ans)		Nombre de morts de leucémie		Risque observé (/10 ⁶ an)	
<u>Radiot.</u>	Nag.	<u>Radiot.</u>	Nag.	<u>Radiot.</u>	Nag.	<u>Radiot.</u>	Nag.
	3		210		11		50
	15		68		2		30
	50		23		0		0
<u>75</u>		<u>5,4</u>		<u>0</u>		<u>0</u>	
	100		23		3		130
<u>150</u>		<u>7,7</u>		<u>1</u>		<u>130</u>	
<u>250</u>		<u>6,6</u>		<u>4</u>		<u>610</u>	
	300		24		15		610
<u>350</u>		<u>8,3</u>		<u>3</u>		<u>360</u>	

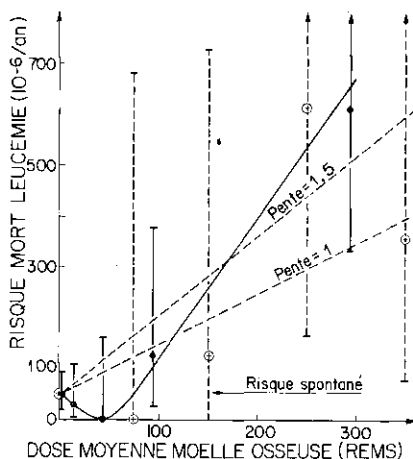


FIG.1. Variation du risque de mort par leucémie quand la dose moyenne reçue dans la moelle osseuse varie. On a rapproché des résultats de Court-Brown et Doll [45], qui avaient observé des rhumatisants traités par des séances d'irradiation limitée au rachis (2 droites et l'intervalle de confiance à 95% de chaque point, sont représentés en tirets), et des résultats de Nagasaki, rapportés par Jablon et Kato [46] (graphe et intervalles de confiance en trait continu).

vérification de cette hypothèse nécessiterait l'observation de groupes d'effectif d'autant plus grand que la dose, ou le coefficient de proportionnalité, sont faibles; selon eux [48] cet effectif devrait atteindre, en personnes-ans : 500 000 pour 20 rems; 2 millions pour 10 rems. Cela signifie qu'après des doses de quelques dizaines de rems, un chercheur ne peut pas trouver d'augmentation statistiquement significative du risque de leucémie dans un groupe irradié, par rapport au groupe témoin, à moins que les effectifs ne soient considérables. En l'absence d'augmentation décelable, la tentation pourrait être grande d'admettre, en pratique, que la dose de seuil d'effet leucémogène est de quelques dizaines de rems. Cela reviendrait à rejeter l'hypothèse de proportionnalité pour de faibles doses; NCRP et CIRP ont estimé que dans l'état actuel de nos connaissances une telle attitude manquerait de prudence [49, 50].

Effectivement, bien que les deux groupes montrent dans le tableau II et sur la figure 1 (limités à des doses pas très élevées) des variations de risque concordantes, on ne peut guère rejeter l'hypothèse de proportionnalité de façon statistiquement significative ($P = 0,06$): les effectifs sont trop faibles.

Cependant, la concordance est d'autant plus remarquable que les deux groupes différaient : l'un ne comprenait que des malades, tous des hommes, irradiés par séances successives de radiothérapie localisée sur le rachis; l'autre, au contraire, comprend des personnes des deux sexes et de tous âges, qui ont subi une irradiation globale et quasi instantanée. Cette concordance est peut-être encore meilleure que ne le représente la figure 1, car en l'absence de donnée nous avons arbitrairement attribué 75 rems à la classe des rhumatisants la moins irradiée, et nous aurions aussi bien pu ne lui en attribuer que 50.

Dans l'un et l'autre des groupes le risque observé passe par un minimum : si on en retranche le risque spontané, de 50, le risque ajouté par l'irradiation est trouvé négatif pour des doses de plusieurs dizaines de rems. Des deux hypothèses, celle d'un risque ajouté négatif [51] suit au plus près les résultats observés; elle est donc probablement plus proche de la réalité que l'hypothèse linéaire de Court-Brown et Doll.

4.2. Cancérogénèse thyroïdienne

La contamination du corps thyroïde retient particulièrement l'attention, surtout pour le nourrisson. Essentiellement à la suite des publications de Hempelmann [11, 52], on craint que son irradiation, même modérée, ne provoque la formation de tumeurs bénignes susceptibles de devenir cancéreuses. Les observations de cet auteur portent sur des personnes dont le thymus ou des végétations adénoïdes ont reçu, dans l'enfance, la prime enfance le plus souvent, des séances de radiothérapie fonctionnelle, cumulant, suivant les cas, d'une à plusieurs centaines de rems; il en a déduit que les coefficients de proportionnalité vaudraient 48 nodules bénins et 5,5 cancers, par rem, par an, et par million d'enfants.

En vue de le vérifier, nous observerons des groupes d'enfants qui ont été contaminés par l'iode des retombées d'explosions nucléaires expérimentales. Dans les flots de Rongelap et d'Utirik [53, 54], dans le Pacifique, début mars 1954, la population, ignorant le danger, s'est contaminée par l'eau des citernes, rationnée en ce début de la saison des pluies. Massive et brève (avant l'arrivée des secouristes) cette contamination a donné des doses qui n'ont pu être évaluées que rétrospectivement, avec une grande incertitude. Par ailleurs, non loin du champ de tir du Nevada, la population a consommé du lait, il est vrai peu contaminé, mais durant des années, cumulant des doses difficiles à évaluer. On a comparé des enfants contaminés ainsi, recrutés dans des écoles de l'Utah et du Nevada, à des enfants arrivés dans ces écoles après la fin des retombées, et aussi à des enfants recrutés à distance, dans l'Arizona [55].

On n'a pas observé d'augmentation du nombre des cancers, même à Rongelap, du moins jusqu'ici, malgré l'importance de la dose, à cause de l'effectif, très faible, et du traitement chirurgical des tumeurs bénignes; aussi dans le tableau III n'avons-nous considéré le nombre prévu que pour ces dernières. Pour chaque groupe irradié ce nombre est la somme (indiquée entre parenthèses) de celui des tumeurs spontanées (calculé d'après les

TABLEAU III. PATHOLOGIE THYROIDIENNE APRES CONTAMINATION ACCIDENTELLE ET GROUPES TEMOINS
(Dose faible ou nulle)

Lieu	Effectif	Durée observ. (ans)	Dose évaluée (rems)	Tumeurs bénignes	
				observées	prévues ^a
Rongelap	19	20	de 900 à 5 000	14 (1 cancer) ^b	de 17 (0,3 + 16,4) à 91 (0,3 + 91,2)
Utah-Nevada	1 378	14	100	18	109 (16 + 93)
Utirik	53	20	de 40 à 80	0	de 3 (0,9 + 2,0) à 5 (0,9 + 4,1)
Utah-Nevada	1 313	14	faible	19 (1 cancer)	16
Arizona	2 140	14	≈ 0	20 (1 cancer)	25

^a Entre parenthèses: nombre de tumeurs spontanées calculé d'après les groupes témoins, et nombre de tumeurs ajouté théoriquement par l'irradiation (d'après Hempelmann: risque = 48 tumeurs/10⁶ enfant-rem-an).

^b Et.2 atrophies du corps thyroïde: risque de lésion considérable.

groupe témoins d'Utah-Nevada et Arizona), et de celui des tumeurs radio-induites (calculé selon Hempelmann); la discordance entre les nombres, observé et prévu, frappante, est statistiquement très significative ($P < 0,001$) à Rongelap si la dose a atteint plusieurs milliers de rems (cela paraît plausible, vu les deux atrophies thyroïdiennes) et dans l'Utah-Nevada. Quant à Utirik, on observe une fois encore un risque ajouté légèrement négatif après une dose modérée. Ceci rappelle l'observation de Conti et coll. [56]: ces auteurs n'ont trouvé aucun cas de cancer dans un groupe de 1564 personnes, âgées d'une vingtaine d'années, et qui avaient reçu, préventivement, systématiquement, 150 rems sur un petit champ centré sur le thymus peu après leur naissance. Cependant, avec un tel effectif, le risque observé, nul, ne diffère de façon statistiquement significative, ni de 500, ni de 2000, risques de leucémie, ou d'autres cancers, observés dans leur groupe témoin.

Malgré tout, ces groupes de personnes irradiées accidentellement, ou systématiquement, montrent que le corps thyroïde est beaucoup moins vulnérable que ne l'ont fait craindre des observations après radiothérapie. L'hypothèse de l'effet nul d'une contamination prolongée, mais faible, et même l'hypothèse d'un risque négatif ajouté par certaines irradiations, paraissent être beaucoup plus proches de la réalité que l'hypothèse linéaire de Hempelmann [52].

4.3. Cancérogénèse in utero

On sait que dans les normes de radioprotection les DMA sont nettement plus faibles pour les femmes en état de procréer que pour les autres travailleurs. Cette rigueur particulière est due à des auteurs dont Alice Stewart apparaît comme le leader [9, 19]: pour eux, de très faibles doses reçues in utero, pour radiodiagnostic, seraient cancérigènes. Mais Jablon et Kato [57] ont infirmé ces conclusions d'après les femmes enceintes irradiées, certaines très fortement, à Hiroshima et Nagasaki; alors, McMahon, dont l'observation [10] avait confirmé celles de Stewart, a fait amende honorable [58]. Il y a déjà une dizaine d'années que Griem, Meir et Dobben [59] avaient signalé l'absence d'effet cancérigène après radiodiagnostic systématique, mais, il est vrai, sur un groupe comprenant seulement un millier de personnes. Il semble bien que ce soit abusivement que de nombreux auteurs ont attribué un tel effet à l'irradiation de personnes qui avaient reçu de très faibles doses, in utero, pour raison sanitaire.

4.4. Tératogénèse

Des médecins conseillent l'avortement thérapeutique si l'utérus, irradié au début de la grossesse, a reçu: 10 rems pour certains, 25 rems pour d'autres. Leur décision repose essentiellement sur les travaux expérimentaux de Rugh [60] sur la souris. Les résultats de cet auteur sont, pour l'essentiel, très schématiquement résumés sur la figure 2. On peut regretter qu'il n'ait pas: distingué les insuffisances pondérales des anomalies morphologiques; irradié à 5 rems; enfin, signalé qu'une dose de 10 rems (qui semble diminuer la probabilité de normalité) pourrait aussi, sans doute, augmenter la probabilité d'implantation utérine. Effectivement, certains radiothérapeutes sont intervenus contre la stérilité.

D'interprétation délicate, l'expérimentation de Rugh vaudrait d'être reprise, ce que nous espérons bien faire faire prochainement.

4.5. Mutagénèse

Les résultats expérimentaux de l'irradiation de générations successives d'animaux n'ont absolument pas confirmé les prévisions alarmantes des calculs. Les raisons vont être exposées ici-même dans une autre communication [61].

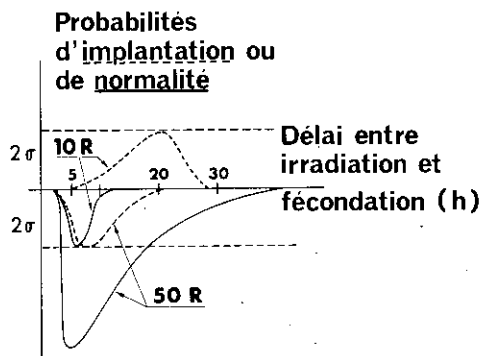


FIG.2. Lorsque l'on fait varier le délai entre irradiation et fécondation (exprimé en heures) on fait varier, par rapport au groupe témoin, les probabilités individuelles moyennes: d'implantation des œufs pondus (graphes en tirets) ou de normalité des fœtus en fin de gestation (graphes en trait continu). Les parallèles à l'axe des abscisses, tracées en tirets, en sont distantes de 2 écarts-types. On n'a rapporté, d'après Rugh [60], que les résultats pour 2 doses (10 ou 50 rems de rayon X en une séance brève) et très schématiquement (voir texte, paragr. 4.4).

5. POUR LIBERER LES CENTRALES NUCLEAIRES

Installations nucléaires les plus nombreuses, et en cours de multiplication, les centrales retiennent l'attention, subissent au plus haut degré le malaise nucléaire, auquel on ne peut espérer remédier qu'en agissant sur les causes.

5.1. A l'origine du malaise

Indéniablement l'énergie nucléaire a complètement raté son entrée sur la scène mondiale; peut-être à Hiroshima (on le croit facilement, trop, sans doute); peut-être à cause de ses présentateurs qui, cependant bien intentionnés, ont parsemé son environnement de risques théoriques de pires maux. La recherche des causes du malaise ne saurait s'en tenir à quelques constatations.

Réuni par l'Organisation mondiale de la santé, un groupe d'experts a, dans un rapport publié en 1957 [62], remarquablement analysé les répercussions psychologiques que pourrait avoir le développement des applications pacifiques de l'énergie nucléaire; en particulier, il a vu là de quoi fixer l'anxiété flottante. Plus récemment Guédeney et Mendel [63], en psychanalistes, ont vu dans les centrales un fixateur de l'angoisse réveillée par le potentiel destructeur des bombes atomiques. D'une façon générale, de nombreux auteurs, dont Freud [64], ont constaté un "malaise dans la civilisation", et essayé de l'interpréter; ainsi, selon Toffler [65], l'accélération de l'évolution des sciences et des techniques ferait subir à l'homme le choc du futur. "Anxiété", "angoisse", "malaise", "choc", ne facilitent pas les échanges entre les parties intéressées, parties d'un ensemble complexe.

5.2. La position des parties dans l'ensemble

Dans une représentation très schématique de la situation (fig. 3), on peut placer au centre les autorités, préoccupées d'assurer au maximum le bien-être de la population par tous les moyens disponibles, mais sollicitées de toutes parts. Trois rectangles séparés correspondent à l'origine des

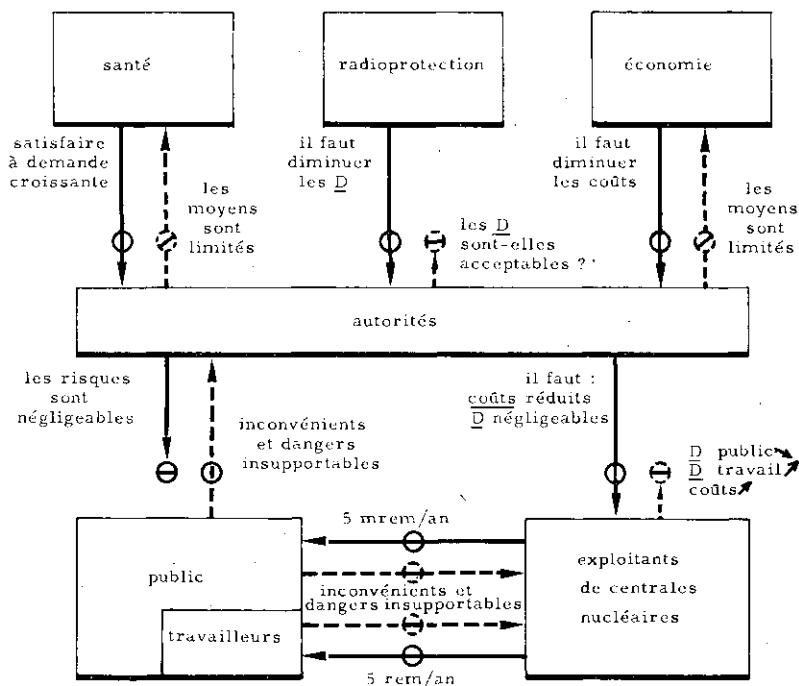


FIG.3. Représentation schématique de la communication entre les parties intéressées par la radioprotection. Suivant qu'elle est ouverte, entrouverte, ou bloquée, la barre dans les ronds est, par rapport à la flèche, de même direction, oblique, ou transversale (voir texte, paragr. 5.2 et 5.4).

sollicitations fondamentales : santé, radioprotection, économie. Les trois flèches qui s'en détachent, tracées en trait continu, montrent le sens des injonctions mentionnées à leur gauche; les deux premières sont antiéconomiques. Les autorités, s'adressant aux exploitants de centrales nucléaires — pour eux seuls — traduisent le tout en une injonction unique : vendre l'électricité au moindre prix ("coûts réduits") mais, surtout, que l'irradiation collective par ces centrales demeure négligeable ("D négligeables"). Pour agir efficacement sur la dose collective, on limite l'irradiation des plus nombreux, celle du public; pour cela, on réduit au maximum l'activité emportée par les effluents, ainsi que la probabilité d'une dispersion incontrôlée, accidentelle, de radioéléments. Malgré tout, informé de l'existence des risques sanitaires (non qualifiés de théoriques) ajoutés par son irradiation, le public proteste, et cela désespère les autorités.

Pour satisfaire une demande sanitaire croissante, pour contenir l'augmentation des coûts, elles ne disposent que de moyens limités. Par ailleurs elles reçoivent des commissions de radioprotection des messages toujours aussi difficiles à traduire en pratique [49, 50]. Dans l'injonction : "réduire les doses autant que possible" (1951), la CIRP, par exemple, a remplacé "que possible", successivement, par : "que cela soit satisfaisant du point de vue pratique" (1959); puis par : "que cela soit réalisable sans difficulté" (1965). Et pour juger de la bonne application de cette injonction sibylline, il est recommandé aux autorités d'obtenir des exploitants, avant chaque opération, l'évaluation, pour la société, des coûts et des profits.

Cette situation fait songer à une "double contrainte".

5.3. Situation de double contrainte

Gregory Bateson et son école [66] ont mis en relief les effets possibles du paradoxe dans une telle situation (double-bind). Nous la décrivons ici très schématiquement en indiquant comment elle peut se créer entre deux parties, se perpétuer, se dénouer.

Pour qu'elle se crée, il faut (p.212): une relation d'autorité entre ces deux parties; que l'activité de l'une conditionne celle de l'autre, et réciproquement; que l'autorité émette une injonction paradoxale (pour lui obéir il faut lui désobéir, de sorte que le message est "indécidable"; on sait que le chien de Pavlov, ne pouvant plus discriminer un cercle d'une ellipse, ce qui était de nécessité vitale, a été atteint de "névrose expérimentale").

Une fois créée elle se perpétue : d'une part parce que le récepteur ne peut se libérer de l'injonction, ni par la critique, ni par le repli (ce serait désobéir), et qu'il serait puni, ou, tout au moins se sentirait coupable, s'il percevait correctement la situation; d'autre part parce que, en retour, son comportement engendre une contrainte pour l'autorité émettrice. Et lorsqu'une double contrainte s'est établie de façon durable, on s'y attend comme à une chose allant de soi (p.216).

L'"indécidabilité" fait que les deux parties ne peuvent dénouer une double contrainte par elles-mêmes. Pour s'en libérer elles doivent exposer leur dilemme à une tierce personne avec laquelle toutes deux ont conservé le mode normal de communication, et "lui demander de prendre la décision de terminer le jeu" (p.238).

5.4. La situation en radioprotection

Retournons à la figure 3. La communication entre les commissions de radioprotection et les autorités ne marche que dans un sens, si bien que ces dernières adressent aux exploitants une injonction paradoxale : multipliez rapidement les centrales nucléaires pour contenir l'augmentation des coûts de l'énergie primaire, mais parce qu'il se pourrait bien qu'il n'y ait pas de seuil pour l'induction d'effets délétères [50], et parce que vous le pouvez, pratiquement, n'augmentez pas sensiblement, pour autant, la dose collective. En diminuant l'irradiation du public, les exploitants augmentent celle des travailleurs, et aussi les coûts; mais les autorités veulent l'ignorer, sous la pression du public qu'inquiète le gigantisme d'installations réputées délétères (gigantisme qui les rend plus économiques). Et comment l'exploitant pourrait-il rassurer ses travailleurs, pour lesquels on tolère une irradiation mille fois supérieure à celle d'un public inquiet ? Tous sachant que "la santé n'a pas de prix", le public se montre de plus en plus exigeant, et les autorités de plus en plus sévères; et les exploitants désespèrent de tirer à bon compte de l'électricité de l'uranium. Ces derniers, comme les autorités d'ailleurs, se sentiraient coupables d'essayer de percevoir correctement une situation où des risques sanitaires arrivent au premier plan. Et les exigences s'accroissent, passant de la santé à l'esthétique, ou autres inconvénients. Mais tant pis pour les travailleurs irradiés, peu nombreux; tant pis aussi pour l'augmentation du prix de revient de l'électricité. Et une telle situation, de plus en plus, semble à tous aller de soi, inévitable, sinon logique.

Tout de même, sans doute pour s'en dégager, les autorités américaines ont, au début de 1975, substitué deux organismes à l'AEC. Peine perdue : la Commission des règlements nucléaires (NRC) s'enferme plus encore. En France, les autorités ont créé, successivement, des organismes de radioprotection, puis de sûreté des installations nucléaires; enfin, peut-être dans l'espoir d'éclaircir la situation, le gouvernement vient de s'adjoindre le Comité interministériel de la sécurité nucléaire, présidé par le premier ministre lui-même. En vain : les difficultés qui partout écrasent l'énergie nucléaire arrivent des Etats-Unis, où elles ont pris naissance, et se développent.

Ces difficultés se propagent suivant un cycle, toujours dans le même sens (des autorités aux exploitants, puis au public, avec retour aux autorités). Par suite du blocage de certaines rétroactions, sous le coup des réactions du public (qui se voit imposer des risques d'affections délétères redoutables, et qui ne comprend pas que l'on puisse qualifier de "négligeables" de tels risques), les autorités ne peuvent qu'ignorer la situation des exploitants. Ce public, c'est tout le monde : on peut le définir, non par des personnes, mais comme un ensemble de réactions affectives individuelles face aux risques de la radioactivité (théoriques, mais effectivement ressentis par tous). Et ces réactions s'amplifient en se conjugant, et aussi à chaque fois que le même cycle se referme. Et on en arrive à préconiser de retirer à l'eau potable une partie de sa radioactivité naturelle [67]. Et toute centrale nucléaire semble, pour le public, frôler sans cesse les circonstances de l'"accident catastrophique" [68].

5.5. Pour renverser la situation

Les deux commissions, NCRP, ou CIRP, parce qu'elles sont investies d'une grandeur morale considérable, et parce qu'elles peuvent émettre des avis autorisés en matière de santé, devraient, après avoir analysé la situation, permettre d'en sortir. Elles le feraient d'autant mieux qu'elles considéreraient l'ensemble du domaine de l'action sanitaire, et si, dans tout ce domaine, pour chacun des effets délétères possibles, elles fixaient, en premier lieu, du moins pour les agents les plus redoutés, une dose de seuil pratique. La voie a été ouverte par Evans [69] et Rowland [70] avec l'un des éléments contaminants les plus redoutables, mais qui, grâce à eux, est le mieux connu, le radium 226, et aussi en génétique, notamment par la revue de Green [71] et par les expériences de Sheridan [72, 73]. Cela permettrait aux autorités d'établir des règlements donnant les limites de pollution admises (en tenant compte, bien entendu, des incertitudes, bien moindres après irradiation que par ailleurs); cela leur permettrait ainsi d'harmoniser l'ensemble des actions curatives, préventives, et de recherche; cela leur permettrait, enfin, de libérer les autorités, les exploitants, et aussi le corps médical (en particulier les radiologues : voir ci-dessus, § 2.3) d'un écrasant, et paralysant, sentiment de culpabilité. Et le public serait rassuré.

6. CONCLUSION

Les commissions de radioprotection, après la Seconde Guerre mondiale, tout en demeurant cantonnées dans leur spécialité, ont étendu leur domaine à l'humanité tout entière. Pour juger de la valeur des normes qu'elles recommandaient, elles ont adopté des références simples : la radioactivité naturelle et les risques les plus courants auxquels la vie moderne expose l'homme.

Valables il y a un quart de siècle, ces références ne le sont plus. On sait aujourd'hui que la radioactivité naturelle est bien au-dessous du seuil pratique pour l'effet délétère le plus sensible : la tératogénèse in utero. Par ailleurs, l'homme n'admet plus de courir des risques imposés, même pour son travail : on doit les lui éviter, à tout prix. Quant aux risques qu'il court ... en procréant, ... par l'avion ou la voiture, ... en se distrayant, ... il les ignore; il préfère qu'il en soit ainsi; l'en avertir sans précaution l'importune et l'inquiète, ou l'irrite.

Le NCRP [50], sans doute, la CIRP, peut-être, le reconnaîtront bientôt, après une analyse psycho-sociologique d'une situation que nous avons essayé de schématiser simplement pour attirer l'attention, non pour la décrire et l'interpréter autant que de besoin. Ensuite, changer les idées, et les habitudes, sera une tâche immense, et difficile : révolutionnaire. Mais salutaire.

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EVALUATION DE RISQUES SOMATIQUES A FAIBLES DOSES

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Abstract-Résumé

EVALUATION OF SOMATIC RISKS ASSOCIATED WITH LOW DOSES.

Epidemiological observations at Nagasaki from 1950 to 1970 suggest that whole-body γ radiation of short duration, where it was not fatal, is not cancerogenic from the statistical point of view for any site except the bone marrow and this only in the category that received an average dose of about 400 rem. It would appear that irradiation with a dose of around 50 rem added a negative risk of leukaemogenesis. In the case of Hiroshima, the interpretation is complicated by the presence of neutrons. It would undoubtedly be of value to compare these observations with those for Nagasaki.

EVALUATION DE RISQUES SOMATIQUES A FAIBLES DOSES.

A Nagasaki, les observations épidémiologiques faites de 1950 à 1970 permettent de conclure que l'irradiation, globale et de courte durée, par rayons gamma, lorsqu'elle n'a pas été mortelle, n'est statistiquement cancérogène pour aucune localisation, sauf pour la moelle osseuse, et seulement dans la classe qui a reçu, en moyenne, environ 400 rem. Il semble bien que l'irradiation à une cinquantaine de rem ait ajouté un risque négatif de leucémogénèse. A Hiroshima, la présence de neutrons complique l'interprétation; il n'en sera que plus intéressant de comparer les observations à celles de Nagasaki.

1. INTRODUCTION

Extraits des observations épidémiologiques publiées en 1971 par Jablon et Kato [1] les risques de cancérogénèse, étudiés d'abord à Nagasaki (où le rayonnement était pauvre en neutrons) le sont ensuite à Hiroshima (où une fraction notable de la dose a été due à des neutrons rapides). Pour terminer, on compare les risques d'Hiroshima à ceux de Nagasaki.

2. LA POPULATION IRRADIEE

En août 1945, les personnes qui se trouvaient à Nagasaki et à Hiroshima ont subi une irradiation globale et quasi instantanée (moins de 10 secondes). D'une façon générale, une personne a reçu une dose d'autant plus grande qu'elle se trouvait plus près de la verticale de l'explosion nucléaire.

Grâce à l'initiative de James V. Neel, généticien américain, l'Académie nationale des sciences américaine et l'Institut national de la santé japonais, ont créé la commission chargée de l'étude des victimes des bombes atomiques (ABCC) [2,3]. Cette commission, avec l'aide de physiciens, a revu chaque cas pour déterminer chaque dose individuelle en fonction, non seulement de la distance au point d'explosion, mais aussi, s'il y avait lieu, de l'interposition d'écrans entre ce point et la personne (toit de maison japonaise, mur de béton, etc...) [4]. Chaque dose individuelle, exprimée en rads dans l'air, est due, en partie à des neutrons (surtout à Hiroshima), et à des rayons γ .

Le dépistage des victimes par l'ABCC a été particulièrement poussé, tant en ce qui concerne la clinique [5] que la nécropsie [6]. Jablon et Kato rapportent les observations faites de 1950 à 1970 ; ils ont réparti chacune des deux populations en cinq classes dosimétriques.

3. LES RISQUES DE CANCEROGENESE

Après avoir exposé en détail comment déterminer la variation du risque de leucémogénèse en fonction de la dose, nous rapporterons la variation de ce risque pour un certain nombre d'autres localisations du cancer, d'abord à Nagasaki, puis à Hiroshima.

3.1. Risque de leucémogénèse à Nagasaki

Pour chaque classe le risque de leucémogénèse est le quotient du nombre de ses leucémiques par son effectif.

On peut considérer, soit les personnes atteintes de leucémie, soit celles qui en sont mortes. Les deux valeurs du risque diffèrent légèrement, pour une même période, en raison de l'existence de formes chroniques. Jablon et Kato ont considéré les morts.

Pour chaque classe le quotient précédent donne, a posteriori, la valeur du risque individuel moyen de leucémogénèse mortelle durant les 21 ans de la période d'observation. Pour simplifier l'expression, nous appellerons cette valeur le risque observé.

En fait, durant cette longue période d'observation, certaines personnes sont mortes, d'autres ont été perdues de vue : l'effectif de chaque classe a varié. Pour ne pas perdre d'information, on compte les effectifs en personnes-ans.¹ Le quotient du nombre des leucémiques morts, par l'effectif ainsi exprimé, donne le risque annuel observé. Ainsi, par exemple, sachant que dans la classe la moins exposée (à moins de 10 rads), on a observé 11 morts par leucémie pour 210 000 personnes-ans, ce risque a été de : $11/210\ 000$.

Pour faciliter l'expression d'un risque, et surtout pour faciliter la comparaison de valeurs différentes, nous exprimons toujours tous les risques en millionnièmes. Dans l'exemple ci-dessus, le risque annuel observé a été de 50 millionnièmes (en nombre arrondi).

Le tableau I montre les limites dosimétriques du découpage des classes ; puis, pour chaque classe : son effectif, en personnes-ans ; le nombre des morts de leucémie ; le risque observé.

Tous les auteurs sont d'accord pour, faute de mieux, prendre comme témoin la classe la moins irradiée. Dans ces conditions, on calcule le risque ajouté par l'irradiation en retranchant le risque observé dans la classe témoin de celui qui l'a été dans chacune des autres. La seconde classe, et aussi la troisième, donnent, pour le risque ajouté, des valeurs négatives.

3.2. Risques de cancérogénèse à Nagasaki

Pour les principales localisations considérées par Jablon et Kato, le tableau II donne : pour la classe témoin, exposée à moins de

¹ Somme des durées d'observation de toutes les personnes de la classe, durées exprimées en années.

TABLEAU I. RISQUES OBSERVE ET AJOUTE DE
LEUCEMOGENESE A NAGASAKI SUIVANT
LA DOSE (1950-1970)

Dose dans air (rads)	10	50	100	200	
Personnes-ans (milliers)	210	68	23	23	24
Morts de leucémie	11	2	0	3	15
Risque observé	50	30	0	130	610
Risque ajouté	0	-20	-50	+80	+560

TABLEAU II. RISQUE AJOUTE DE CANCEROGENESE
POUR DIVERSES LOCALISATIONS A NAGASAKI,
SUIVANT LA DOSE (1950-1970)

Dose air (rads)	*	10	50	100	200
Moelle osseuse	50	- 20	- 50	+ 80	+ 560
Arbre respiratoire	140	- 35	- 90	+ 35	+ 70
Estomac	550	+ 90	+ 20	- 200	+ 30
Tractus intestinal	970	+ 50	+380	- 540	+ 260
Sein (femmes)	40	+ 50	+ 50	+ 50	+ 30

* Valeurs prises pour référence, tenant lieu de risque spontané

10 rads, les valeurs du risque observé (elle tient lieu de risque spontané); pour les autres classes, les risques ajoutés, calculés comme pour la leucémie.

3.3. Risques de cancérogénèse à Hiroshima

Comme nous l'avons fait dans le tableau II pour Nagasaki, nous rapportons dans le tableau III les valeurs du risque de cancérogénèse pour les mêmes localisations.

4. DISCUSSION

Sur les deux populations réparties en classes suivant les mêmes limites dosimétriques, les risques de cancérogénèse ajoutés par l'irradiation évoluent en fonction de la dose de façon tout à fait différente.

Il est vrai que leur irradiation diffère : alors que la quasi-totalité de la dose est due au rayonnement γ à Nagasaki, une fraction notable provient de neutrons à Hiroshima. Le tableau IV donne quelques points de repère, à titre indicatif, d'après Auxier et coll. [7].

TABLEAU III. RISQUE AJOUTE DE CANCEROGENESE
POUR DIVERSES LOCALISATIONS A HIROSHIMA,
SUIVANT LA DOSE (1950-1970)

Dose air (rads)	* 10	50	100	200	
Moelle osseuse	40	44	100	290	970
Arbre respiratoire	140	70	100	150	150
Estomac	900	40	120	290	230
Tractus intestinal	1400	0	140	340	390
Sein (femmes)	50	16	33	150	60

* Valeurs prises pour référence, tenant lieu de risque spontané

TABLEAU IV. DOSIMETRIE DANS L'AIR (rads)
A HIROSHIMA ET NAGASAKI

Distance (mètres) :		800	1200	2000
Hiroshima	n :	480	50	0,6
	Y :	710	100	2
Nagasaki	n :	170	20	0,3
	Y :	2100	400	15

Allons d'abord à Nagasaki, où l'irradiation a été plus simple, puis à Hiroshima. Pour terminer, nous essaierons, après Jablon [8], de superposer les résultats des deux observations.

4.1. Variation du risque de cancérogénèse à Nagasaki

L'ensemble des 4 dernières colonnes du tableau II suggère deux remarques.

1°) Sur les deux premières lignes, le risque ajouté par l'irradiation décroît d'abord quand la dose augmente. Il décroît par valeurs négatives ; en d'autres termes, une irradiation globale et quasi instantanée, pour une dose comprise entre 10 et 100 rads, exercerait une action anticancérogène sur la moelle osseuse et sur l'arbre respiratoire ; il nous semble que ces valeurs négatives méritent de retenir toute l'attention des chercheurs, plutôt que d'être qualifiées de "paradoxaes", et laissées hors discussion.

2°) Un seul risque ajouté par l'irradiation diffère de zéro de façon statistiquement significative : la moelle osseuse, l'un des organes les plus sensibles, n'a montré de réaction pathologique significative que pour une irradiation globale, quasi instantanée, à une dose de 400 rads (moyenne de la classe). Le tractus gastro-intestinal ne semble avoir

réagi à l'irradiation, ni dans un sens, ni dans l'autre : les valeurs de la troisième ligne, et de la quatrième, fluctuent autour de zéro de façon qui paraît tout à fait aléatoire. Quant au sein de la femme, le fait que le risque demeure constamment légèrement positif pourrait être dû à une valeur de référence (classe irradiée à moins de 10 rads) statistiquement trop faible.

4.2. Variation du risque de cancérogénèse à Hiroshima

Le tableau III ne contient que des valeurs positives. Examinons-le comme nous venons de le faire pour le tableau précédent.

1°) Sur les deux premières lignes, le risque ajouté par l'irradiation croît d'emblée ; pour une dose comprise entre 10 et 50 rads, il diffère déjà de zéro de façon statistiquement significative (au risque de 5 %). Tandis que dans la moelle osseuse le risque ne cesse de croître avec la dose, pour devenir considérable au-delà de 200 rads, il demeure étale dans les voies respiratoires à partir d'une cinquantaine de rads : la cancérogénèse serait-elle déjà saturée ? On sait en effet que de très fortes doses sont moins cancérogènes que des doses moyennes.

2°) Sur les trois dernières lignes aucun risque ajouté par l'irradiation ne diffère de zéro de façon statistiquement significative.

4.3. Essai de superposition des résultats d'Hiroshima et de Nagasaki

On sait que la Commission internationale des unités et des mesures de radiologie (CIUMR, en anglais: ICRU) écrit [9]:

$$\underline{H} = \underline{D} \underline{Q} \underline{N}$$

où l'équivalent de dose, \underline{H} , est le produit de la dose absorbée, \underline{D} , par le facteur de qualité, \underline{Q} , et par \underline{N} , qui représente tout autre facteur de modification.

En vue d'éviter toute ambiguïté, marquons d'un indice γ , ou n , les grandeurs dosimétriques qui sont relatives aux rayons γ , ou aux neutrons. On peut écrire, puisque $\underline{Q} = 1$ pour les rayons γ :

$$\underline{H}_{\gamma} = \underline{D}_{\gamma} \quad (1)$$

$$\text{et} \quad \underline{H}_n = \underline{Q} \underline{D}_n \quad (2)$$

Admettons, dans un premier temps, avec Harald Rossi [10] que le facteur de qualité des neutrons varie en fonction de la dose absorbée suivant la relation :

$$\underline{Q} = 44 / \sqrt{\underline{D}_n} \quad (3)$$

Alors, d'après (2) et (3) :

$$\underline{H}_n = 44 \sqrt{\underline{D}_n} \quad (4)$$

Pour l'instant, comme Jablon [8], nous ne considérerons que le risque de leucémie.

On ne saurait mettre en parallèle deux classes de personnes qui ont reçu la même dose absorbée lorsque, pour l'une d'elles, une fraction notable de cette dose est due à des neutrons. Il serait, par

TABLEAU V. RISQUE AJOUTE DE LEUCEMIE,
ETABLI A PARTIR DU RAPPORT BEIR

Classes (rads) [BEIR]	Hiroshima				Nagasaki			
	rads \bar{D}_Y	rems \bar{D}_n	risque $\bar{H}_Y + \bar{H}_n$	ajouté	rads \bar{D}_Y	rems \bar{D}_n	risque $\bar{H}_Y + \bar{H}_n$	ajouté
5 - 19	8	2	70	+ 20	10	0	10	- 10
20 - 49	20	5	120	+ 180	31	0	30	- 40
50 - 99	57	13	220	+ 150	70	0,2	90	- 40
100 - 199	108	30	350	+ 390	144	1,4	200	+ 130

contre, intéressant d'attribuer le même équivalent de dose à deux classes, l'une d'Hiroshima, l'autre de Nagasaki, lorsqu'elles montrent le même risque ajouté, ou à peu près. Mais il faudrait pour cela subdiviser la classe la moins irradiée à Hiroshima, ce qui serait fort possible (à condition de disposer du fichier) parce qu'elle est beaucoup plus nombreuse qu'à Nagasaki (800 000 personnes-ans au lieu de 210 000) [1, pp. 61, 67]. Faute de mieux, en attendant, nous n'avons pu que nous reporter au "rapport BEIR" [11, p. 102]. Mais ce rapport considère les leucémies déclarées, non les morts ; de plus, il correspond à une observation qui s'arrête en 1966 ; enfin, et surtout, il ne distingue que 3 classes (au lieu de 2, il est vrai) entre 0 et 50 rads. Nous avons néanmoins, à l'aide de ses données, établi le tableau V.

On voit dans ce tableau que la formule de Rossi est tout à fait valable pour rapprocher la classe (50-99) rads d'Hiroshima, soit, en moyenne, 220 rems, de la classe (100-199) rads, soit, en moyenne, 200 rems, de Nagasaki : le risque de leucémie y a même valeur (150 ou 130 millionièmes).

Mais une dose de 200 rems ne peut être reçue en quelques secondes qu'accidentellement.

Pour pénétrer dans le domaine d'irradiations pas trop fortes, il faudrait subdiviser la classe (0-5) rads à Hiroshima ; c'est encore possible parce qu'elle comprend 3 fois plus de personnes que celle de Nagasaki. Au cas où l'on retrouverait, dans l'une ou dans plusieurs des sous-classes, un risque négatif, il faudrait donner à \bar{Q} des valeurs sensiblement supérieures à celles que donne la formule de Rossi, puisque la classe (0-5) rads d'Hiroshima devrait recouvrir toutes les classes de Nagasaki qui sont comprises entre 0 et plus de 100 rads.

5. CONCLUSION

A Nagasaki, où le rayonnement comprenait relativement peu de neutrons, avec 25 ans de recul, l'irradiation, globale, quasi instantanée, ne s'est encore avérée statistiquement cancérogène pour aucune localisation, sauf pour la moelle osseuse, et seulement dans la classe la plus atteinte (dose proche de 400 rads). Donc une irradiation globale par rayons γ , si elle n'a pas été mortelle à brève échéance, ne pourrait s'avérer, à long terme, que leucémogène.

La variation du risque de leucémogénèse semble même confirmer notre hypothèse selon laquelle une irradiation de caractéristiques convenables ajouterait un risque négatif (ici, pour les doses de moins de 100 rads).

Nous souhaitons que la population d'Hiroshima soit répartie en classes dosimétriques plus étroites, d'effectif comparable aux classes de Nagasaki, particulièrement dans le domaine des doses pas trop fortes. Cela permettrait peut-être de trouver aussi à Hiroshima une ou plusieurs classes à risque de leucémogénèse négatif.

Les 3 publications dont nous disposons donnent des valeurs numériques quelque peu différentes pour les doses de neutrons. Cependant dans le domaine des doses pas trop grandes la précision est primordiale, d'autant plus qu'aux doses de quelques rads, ou moins, le facteur de qualité pourrait être beaucoup plus grand encore que ne l'indique la formule de Rossi.

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DISCUSSION

(on the previous two papers)

G.W. BEEBE: As regards the Nagasaki data on leukaemia, it should be noted that those published and quoted are very few, because in our cohort approach at the Radiation Effects Research Foundation (formerly Atomic Bomb Casualty Commission) we have been using only a fraction of the data. Not only are the published data on leukaemia subject to very large sampling errors but, when the total leukaemia registry of Nagasaki is examined and studied in the light of the total population of survivors in the city, not merely those in the cohort, the dip in incidence seems to disappear and the curve looks much more like that for Hiroshima. These observations will shortly be offered for publication, as the numerical weakness of the published Nagasaki data is sometimes lost sight of; they should not be taken as strong evidence, by themselves, of curvilinearity.

M. DELPLA: I have worked with the only results which I had available, and I feel that the interpretation I have suggested, which is a simple working hypothesis for the time being, deserves attention.

In this connection, I must emphasize the importance of dividing the population of Hiroshima on the basis of dose (rads in air) into a greater number of categories than that of Nagasaki.

DU RISQUE GENETIQUE AUX FAIBLES DOSES

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Abstract-Résumé

THE GENETIC RISK AT LOW DOSES.

How are we to assess the genetic risk to man resulting from exposure to low doses of irradiation from the environment of nuclear power plants? The team of Brewen and Preston showed that there was a decrease by a factor of four in the rate of chromosomal aberrations existing in germinal cell line as compared with somatic cell (leukocyte) line. They subsequently demonstrated the importance of the selection process taking place at the time of gametogenesis. However, their study went only as far as a 50-rem dose. Applying fractionation of a 275-rem dose (55 daily sessions of 5 rem each), Sheridan was unable to induce in mice either dominant or recessive mutations in quantities greater than in the controls: the recessives would appear to have been fewer, but the difference was not significant. Nor did experiments by various teams involving systematic irradiation (either chronic or acute) of several tens of generations of mice and rats succeed in producing mutants over the different generations. In order to affect fertility and also mortality before weaning, it is necessary to achieve single doses of the order of 500 rem. Results of this kind, which show the existence of both repair and selection, are reassuring, although they are obtained at dose rates that have nothing in common with those which are relevant to the environment. They support the assumption that no deleterious effect is present in the latter case.

DU RISQUE GENETIQUE AUX FAIBLES DOSES.

Où situer le risque génétique chez l'homme, par suite de l'exposition aux faibles doses dispensées par l'environnement des centrales nucléaires? L'équipe de Brewen et Preston a mis en évidence la diminution d'un facteur quatre du taux d'aberrations chromosomiques existant dans la lignée germinale, par rapport à la lignée somatique (leucocytes). Ces chercheurs ont ensuite montré l'importance de la sélection intervenant lors de la gamétogénèse. Mais leur étude s'arrête à 50 rem. Sheridan, en fractionnant 275 rem en 55 séances quotidiennes de 5 rem chacune, n'a pu induire chez des souris des mutations ni dominantes, ni récessives en quantités supérieures à celles des témoins: les mutations récessives seraient plutôt inférieures (mais la différence n'est pas significative). Des expérimentations, effectuées par différentes équipes, d'irradiation systématique soit chronique, soit aiguë, d'une ou plusieurs dizaines de générations de souris et de rats, n'ont pas non plus réussi à générer des mutants sur les différentes générations. Il faut atteindre des doses uniques de l'ordre de 500 rem pour parvenir à affecter la fertilité et aussi la mortalité avant sevrage. De tels résultats, montrant à la fois l'existence de la restauration et de la sélection, sont rassurants, bien qu'obtenus à des doses et des débits de dose sans commune mesure avec ceux intéressant l'environnement. Ils autorisent à penser qu'aucun effet délétère ne doit exister dans ce dernier cas.

1. INTRODUCTION

Cancérogénèse et mutagénèse ne seraient que les deux facettes du même type d'effet élémentaire: la lésion de l'ADN cellulaire. En effet, nombre de substances chimiques hautement cancérigènes présentent un effet mutagène aussi bien "in vivo" qu'"in vitro" [1, 2, 3, 4]; d'autre part l'effet cancérigène des U.V. sur la peau serait consécutif à un défaut de réparation des mutations créées au niveau de l'ADN et plus fréquent par conséquent chez ceux présentant une déficience enzymatique (xeroderma pigmentosum) [5, 6].

A l'origine, on aurait donc, ou une mutation génique, ou une malformation chromosomique. Il est très intéressant de comparer l'importance relative du dommage ainsi créé, pour la même irradiation, sur les lignées cellulaires somatiques ou germinales.

Dans le domaine de la génétique, plus particulièrement, nous nous proposons d'examiner, à la lumière des résultats expérimentaux sur mammifères, quelle peut être l'importance constatée du risque au niveau des faibles doses.

2. COMPARAISON D'EFFETS SOMATIQUES ET GENETIQUES

Une étude très instructive a été menée sur l'induction comparée d'aberrations chromosomiques sur des lignées cellulaires somatiques et germinales. Heddle ayant remarqué que les aberrations asymétriques comme les dicentriques et les aberrations symétriques, telles que les translocations, étaient induites au même taux par une irradiation donnée [7], Brewen et Preston ont ensuite comparé, sur différentes espèces animales (souris, hamsters, cochons d'Inde, ouistitis) puis sur l'homme, le taux de dicentriques obtenus sur les leucocytes circulant au taux de translocations induites dans les spermatogonies, et mesuré au stade spermatocytes I [8]. Aux doses voisines de 100 rems, un rapport de quatre a été trouvé sur la souris et de deux sur l'homme ; à 200 rems, chez l'homme, le rapport prend la valeur de 5 [9].

Selon les mêmes auteurs, s'appuyant sur les résultats sur les souris de Ford et de Lyon [10, 11], pour 600 rems, seulement la moitié des mutations prévisibles se retrouve sur la descendance, mutations se concrétisant par des morts "in utero" ou par la naissance d'hétérozygotes semi-stériles. Ces derniers représentent finalement 12,5 % des translocations notées au stade spermatocyte (soit le quart de la moitié) d'où seulement 3 à 4 % des dicentriques repérés dans les leucocytes du géniteur mâle. (cf figure 1)

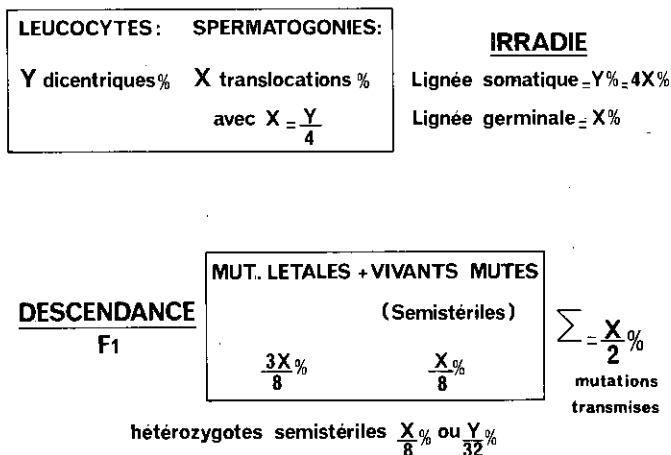


FIG. 1. Transmission des mutations létales dominantes (translocations symétriques radio-induites sur les spermatogonies) — D'après les travaux de Brewen et Preston [8].

Etendant ces résultats à l'homme, les auteurs en déduisent que si 100 rems de rayons X (administrés en dose unique et à fort débit de dose) induisent 14 % de dicentriques sur les leucocytes, ils devraient conduire à l'apparition de 0,3 à 0,5 % de transloqués équilibrés à la 1ère génération.

Mais cette prévision ne peut être étendue aux très faibles doses, l'observation s'arrêtant à 50 rems. Le mérite de cette étude est d'abord d'avoir mis l'accent sur l'importance des phénomènes de sélection intervenant lors de la spermatogénèse, puisque 50 % des aberrations constatées au stade spermatocyte I ont disparu au stade spermatozoïde mûr.

Il est probable que la sélection naturelle qui intervient déjà spontanément chez l'homme, et qui se manifeste par une perte cellulaire de plus de 30 %, au cours des stades de maturation de la spermatogonie en spermatozoïde, doit pouvoir encore s'intensifier sous l'effet d'une irradiation [12].

Le second mérite de l'étude de Brewen et Preston a été de montrer que le risque génétique, à dose égale, était moindre que le risque somatique.

Or existe-t-il un quelconque risque somatique pour des doses de l'ordre de 10 rems, pris en une fois et à fort débit de dose ? Aucun effet délétère à long terme n'a pu être relevé à ce niveau de dose. Comment se pourrait-il donc qu'il existât un risque génétique, dans le même domaine de dose ?

Nous allons examiner rapidement les différents points de vue et l'évolution des idées en la matière.

3. BASES DE L'APPRECIATION DU RISQUE GENETIQUE : LEUR EVOLUTION

3.1. Anciennes données

Après la découverte des mutations radio-induites sur la drosophile par Muller en 1927, différents travaux effectués par le même auteur et d'autres, sur le même insecte, ont conduit à affirmer les faits suivants [13] :

- il existe une relation linéaire et sans seuil entre la fréquence des mutations et la dose de rayonnement.
- la répartition de la dose dans le temps n'a pas d'influence, et notamment le débit de dose,
- il y a additivité des effets d'irradiations successives (cumul sur la vie de l'individu et cumul au cours des générations successives),
- il n'y a pas de faculté d'adaptation.

Ce sont là, comme le dit Lejeune en 1961 [14] : "les bases fondamentales sur lesquelles reposent les prévisions théoriques généralement présentées".

Sur ces bases, le risque de mutations à l'unité de dose a été déduit de celui observé à forte dose et à fort débit de dose. On a procédé de même à la suite d'expérimentations sur mammifères, puis ce principe de calcul a été étendu à l'homme, en tenant compte du nombre de gènes supérieur du génome humain : ainsi des valeurs de risque ont été avancées, révisées depuis.

3.2. Evolution et position actuelle

A partir de 1958, suite aux travaux de Russell et Kelly [15], on retint que la chronologie était un paramètre important à prendre en considération.

D'autres travaux de Russell, dont les conclusions furent rassemblées dans sa communication à Genève en 1971, [16] puis ceux de Lyon, Sheridan, Searle, Preston, Brewen etc. permirent de réviser les données d'évaluation du risque aux faibles doses. Celui-ci fut estimé bien inférieur à celui déduit des fortes doses. Le groupe de l'UNSCEAR a proposé des valeurs correspondantes à l'irradiation chronique (à faibles débits de dose) ou à l'irradiation unique à faibles doses pour l'homme. Nous reproduisons au tableau I l'essentiel de leurs conclusions [17].

Ce tableau incite à faire trois remarques :

- 1°) Pour ce type d'irradiation, seul est pris en compte le risque inhérent à l'irradiation du mâle, l'ovaire s'étant révélé susceptible de restauration totale aux faibles débits de dose (Russell).
- 2°) Une sélection très importante intervient, à la fois lors de l'évolution des spermatogonies porteuses des mutations ponctuelles récessives et aussi de celle des conceptions où des translocations ont été induites.
- 3°) Les données sont déduites de celles obtenues sur la souris, à l'exception de celles de la dernière ligne, calculées en adoptant la valeur de 100 rads pour la dose doublante. On voit qu'il existe un facteur 5 entre les deux.

Le Comité du BEIR, lui, a fixé les limites 20 à 200 rems pour la dose doublante. Il a calculé l'importance du dommage induit sur une population irradiée à la DMA de la CIPR, c'est-à-dire à 5 rems par génération : les résultats sont donnés au tableau II. Les valeurs transcrites correspondent à l'hypothèse selon laquelle la dose doublante se situerait à 200 rems, valable pour les conditions d'irradiation les moins sévères [18].

Aux D. M. A., le risque ajouté à la première génération ne serait plus que le millième du taux spontané, mais, bien que faible, il n'est plus négligeable, dès qu'on l'applique à des populations numériquement importantes, telle celle des Etats-Unis. En France, compte-tenu du nombre de naissances vivantes par an ($\approx 800\ 000$), l'irradiation aux normes devrait selon ces bases conduire annuellement encore à 48 mutés à la première génération et 240 mutés à l'équilibre.

Mais, en réalité, que faut-il penser de ces valeurs ?

3.3. Discussion

Examinons ce qui demeure, parmi les assertions établies à l'origine et énoncées au chapitre 2.

3.3.1. Relation linéaire dose-effet

La relation linéaire dose-effet, souvent confirmée dans le domaine des fortes doses, s'est par contre trouvée infirmée pour les doses inférieures à 200 rems.

C'est ainsi qu'Edwards et Searle, de même que Russell, irradiant les oocytes de souris, n'ont pas retrouvé aux doses les plus faibles de leur étude les résultats escomptés, en appliquant la loi de proportionnalité aux valeurs obtenues à fortes doses [19, 20]. L'irradiation des oocytes de cochon d'Inde

TABLEAU I. RISQUE GENETIQUE CHEZ L'HOMME, A L'UNITE DE DOSE, POUR LES FAIBLES DOSES OU LES TRES FAIBLES DEBITS DE DOSE

(Tiré de l'UNSCEAR, tableau 29, p.289)

Type de mutation	Taux prévisionnel/10 ⁶		
	Spermatogonies	Oocytes	Conceptions (F ₁)
Mutations ponctuelles et récessives	1500 ^a (36) ^b	très bas	30 - 75 (1 - 2) ^b
Dominantes visibles	2		2
Du squelette	4		
Translocations réciproques ^c	15	très bas	2 malformations congénitales + 19 pertes précoces d'embryons + 9 avortements reconnus
Pertes de chromosomes X	très bas	8	8 pertes précoces d'embryons ou avortements ^c
Autres anomalies chromosomiques	très bas		très bas
Domimage génétique total	1521 (57) ^d		
Domimage génétique théorique	300 ^e		6 - 15 ^e

^a Estimation d'après les résultats obtenus sur les locus spécifiques chez la souris.

^b Estimation d'après le taux de récessifs létaux induits sur le génome des spermatogonies de souris.

^c Données pour l'irradiation X à faible dose; les valeurs applicables à l'irradiation γ devraient être divisées par 2.

^d Obtenu en additionnant 36+2+4+15 dans la colonne: Σ des mutations létales.

^e Relatif à l'incidence spontanée d'anomalies génétiques constatées parmi les naissances vivantes, en admettant une dose doublante de 100 rad.

TABLEAU II. DOMMAGE GENETIQUE ESTIME POUR 5 rem PAR GENERATION ET PAR MILLION DE NAISSANCES VIVANTES
(Tiré du rapport BEIR, tableau 4, p.57)

Type d'anomalie	Incidence normale	Effet de 5 rem/génération	
		1 ^{ère} génération ^a	Equilibre ^a
Maladies dominantes	10 000	50	250
Maladies récessives et chromosomiques	10 000	relativement bas	
Anomalies congénitales	15 000		
Anomalies retardées	10 000	5	50
Maladies constitut. et dégénératives	15 000		
Total	60 000	60	300

^a L'évaluation est faite en considérant une dose doublante de 200 rem.

et de hamster a conduit Lyon et Smith à noter une diminution de la mortalité in utero par rapport aux témoins, à 70 rems pour le cochon d'Inde et à 200 rems pour le hamster [21]. L'irradiation à fort débit de dose de la souris mâle n'a pas permis non plus à Searle de vérifier la loi linéaire, entre 400 et 56 rems [22].

Il n'existe pas d'étude aux doses inférieures à 50 rems. Néanmoins celle concernant le fractionnement des doses est instructive à ce sujet (voir 3.3.2.2.).

De toute façon, implicitement, le Comité du BEIR a prévu une diminution d'un facteur 10 du risque, pour les faibles doses, ayant fixé les limites de la dose doublante à 20 à 200 rems, selon la sévérité des conditions de l'irradiation.

3.3.2. Influence de la chronologie

Deux paramètres concernant la chronologie sont à retenir : la vitesse avec laquelle la dose est administrée, c'est-à-dire le débit de dose, ainsi que le mode de répartition de la dose dans le temps, c'est-à-dire le fractionnement éventuel de la dose et le nombre de séances ainsi que l'intervalle de temps ménagé entre les séances.

3.3.2.1. Débit de dose

A la suite de Russell [23], de nombreux chercheurs ont trouvé que le débit de dose jouait un rôle important en génétique. Cet effet est encore plus sensible sur les mutations chromosomiques que sur les mutations ponctuelles. En effet Searle a pu noter qu'à 600 rems, le taux de translocations induites par les rayons γ sur les spermatogonies de souris était réduit d'un facteur voisin de 10 pour un abaissement de 4000 fois du débit de dose [24]. Au débit de dose le plus bas (0,08 rem/min), le taux de translocations est deux fois plus faible avec les rayons γ qu'avec les rayons X [24]. La relation entre le débit de dose et le pourcentage de cellules affectées par la mutation serait, d'après Searle, du type exponentiel [24]. Nous reproduisons sur la figure 2 les résultats obtenus par cet auteur. Cette figure montre qu'il doit exister un domaine de débit de dose pour lequel l'effet s'annule.

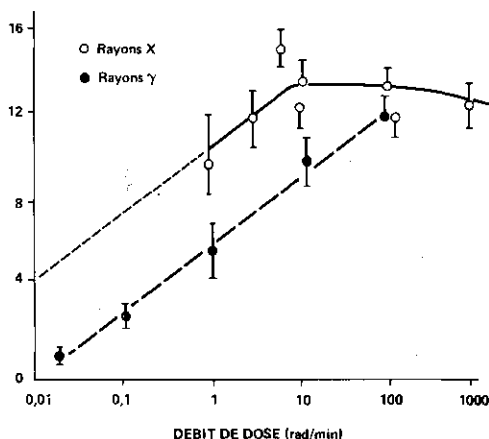


FIG.2. Fréquence des aberrations chromosomiques (translocations mesurées sur les spermatocytes), selon le débit de dose, pour deux types d'irradiation: X (cercles vides) et γ (cercles pleins) — D'après les travaux de Searle et al. [24].

3.3.2.2. Fractionnement de la dose

Selon les cas, le fractionnement de la dose peut ou être sans effet, ou exalter l'effet ou le réduire.

C'est ainsi que 1000 rems administrés en 2 séances de 500 rems sont équivalents à 1000 rems en une fois pour un intervalle de temps de 2 h ; et si l'intervalle est de 24 h, on constate une augmentation d'un facteur 5 du taux de mutations aux locus spécifiques, selon Russell [25], ainsi que du taux de translocations induites sur les spermatogonies, selon Lyon et Morris [26]. Le fractionnement en séances quotidiennes de 200 rems amène encore une augmentation de l'effet, un peu moindre, toutefois. Les auteurs expliquent ce phénomène par la diminution de l'effet létal aux doses fractionnées, s'accompagnant d'une augmentation de l'effet mutagène, et aussi d'une sensibilisation des cellules amenées à synchroniser leur développement et à se trouver en plus grand nombre en même temps à un stade vulnérable. Mais il en est tout autrement pour les faibles doses répétées. En effet, Lyon et Coll. ont trouvé un abaissement très significatif du taux de translocations induites sur les spermatogonies par le fractionnement de 300 rems en, soit 5 fois 60 rems, soit 30 fois 10 rems, soit, mieux, 60 fois 5 rems [27]. Dans ce dernier cas, ils notent encore 1,3 % d'anomalies (cellulaires) au lieu de 6,3 %. Mais il s'agit là de l'effet primaire, qui, comme l'ont montré Brewen et Preston, se trouve ensuite notablement réduit lors de la gamétogénèse et de la fécondation. Une expérimentation complète sur la descendance des souris a été menée par Sheridan, concernant un même mode de fractionnement : 275 rems fractionnés en 55 séances quotidiennes de 5 rems (à l'aide de rayons X et à 75 rem/min) [28]. La recherche des mutations létales dominantes n'a pas permis de déceler de différence avec les témoins. Quant aux mutations récessives létales, recherchées par accouplements en back-cross, non seulement elles n'ont pas augmenté et même elles semblent inférieures à celles des témoins (3,8 % contre 5,5 %) : la différence n'est cependant pas statistiquement significative.

Ainsi Sheridan conclut que "l'administration d'une dose d'irradiation en de nombreuses petites fractions ne conduit pas à une élévation de la fréquence des mutations".

TABLEAU III. ETUDES D'IRRADIATION CHRONIQUE DE SOURIS ET DE RATS SUR PLUSIEURS GENERATIONS

Auteurs	Espèce	D (rem/j)	n gén.	D cumul. (rem)	Critères de l'étude ^a		
					Long.	Poids	Fécond.
Brown [29]	rat	2	12	3936	0	0	0 légèrement - à la 6 ^e gén.
Stadler et Gowen [30]	souris (M + F)	2,6	10	1580	0	0	0
Searle [31]	souris	1/nuir	30	4800	0	0	0 - après 24 gén.

^a Les symboles utilisés sont: 0 pour aucune modification; + si augmentation; - si diminution.

TABLEAU IV. ETUDES D'IRRADIATION AIGUE DE SOURIS MALES SUR PLUSIEURS GENERATIONS

Auteurs	D ^a (rem)	Ḋ (rem/min)	Délai fécond.	n gén.	D cumul. ^b (rem)	Critères de l'étude ^c		
						Long.	Poids	Fécond.
Frøien [32]	500 (G)	> 20	1 - 7 j	2	500	0		-
Schlager et al. [33]	50, 100 (L)	90	7 sem.	7 - 10	250 - 500	0	0	0
	900	90	7 sem.	6	2700	0	-	0
King [34]	545 (G)	16	12 sem.	5	1360	0	+	-
Green [35]	50, 100 (L)	90	7 sem.	12 - 15	300 - 375 et 600 - 750	0	0	0
Newcombe et McGregor [36, 37]	600 (L)	> 20	1 - 18 j	13	3900	0 avant sevrage	(13 ^e gén.)	-
Spalding et Brooks [38, 39]	200 (G)	45 - 50	1 - 8 j	45 - 50	4500 - 5000	0	0	légèrement - à la 6 ^e gén.

^a (G) = irradiation globale; (L) = irradiation locale, aux gonades.

^b La dose cumulée a été calculée, selon le nombre de générations et en divisant la dose par deux, car seul le géniteur mâle a été irradié, à chaque génération.

^c Les symboles suivants ont été utilisés: 0: pas de différence significative avec les témoins; +: augmentation significative par rapport aux témoins;

-: diminution significative par rapport aux témoins.

3.3.2.3. Cumul des doses

Il va de soi que si l'abaissement du débit de dose et le fractionnement poussé de la dose conduisent à une diminution substantielle de l'effet, il ne peut y avoir cumul des effets des petites doses au cours de la vie de l'individu.

Mais qu'en est-il du cumul au cours des générations ?

L'étude de Sheridan a montré que, même pour 275 rems en dose unique, les mutations récessives létales ne dépassaient pas statistiquement celles des témoins.

D'autres expérimentations ont délivré des doses sur une ou plusieurs dizaines de générations : elles permettent d'apprécier les risques de cumul sur n générations.

a) Irradiation systématique et chronique

Le tableau III rassemble les principales expériences d'irradiation chronique γ menées sur une ou plusieurs dizaines de générations de souris et de rats, dont seul le mâle a été irradié, sauf dans l'expérience de Stadler et Gowen où les deux géniteurs ont subi le même traitement. Les critères de l'étude ont été la longévité des générations successives, le poids moyen des individus et la fécondité.

L'ensemble des résultats est négatif, sauf en ce qui concerne la fécondité, légèrement réduite à la 6ème génération chez les rats et à la 24ème chez les souris. Mais il s'agit de débits de dose encore très élevés, de 70 à 200 fois supérieurs à ceux concernant les irradiations maximales des groupes humains les plus exposés (travailleurs). Si l'on compare aux doses maximales admissibles pour la population, on trouve un facteur de 2000 à 5000.

Les résultats négatifs obtenus ici permettent a fortiori de ne prévoir aucun effet génétique délétère au niveau des D.M.A., ni aucun cumul aussi bien sur la vie de l'individu, que sur les générations successives.

b) Irradiations aiguës systématiques

De nombreuses autres expériences ont porté sur une irradiation unique du mâle, soit localement sur les gonades soit sur l'organisme entier, à des doses variables, ayant atteint 600 et même 900 rems dans certains cas. L'intervalle de temps entre l'irradiation et l'accouplement a varié d'une expérience à l'autre, ce qui permet d'évaluer et de comparer les risques inhérents aux mutations induites dans les formes matures des spermatozoïdes à celles des spermatogonies.

Les principaux résultats sont collationnés sur le tableau IV. On ne peut y relever aucun effet génétique délétère pour les doses inférieures ou égales à 200 rems, quelque soit l'intervalle de temps ménagé avant la fécondation. Aux doses plus élevées, une atteinte de la fécondité peut être notée, résultant de l'apparition d'hétérozygotes semi-stériles (Frölen à 500 rems, King à 545 rems, Newcombe et Mc Gregor à 600 rems). Néanmoins, même à 600 rems, la longévité de la descendance vivante n'a pas été modifiée, à ceci près qu'un taux de décès supérieur à la normale a pu être noté chez les petits avant sevrage. Cette constatation faite par Newcombe et Mc Gregor a été confirmée par Pomerantzeva[40]. La période avant sevrage constitue donc la dernière étape de la sélection.

4. CONCLUSION

La mise en évidence expérimentale de l'influence énorme jouée par la chronologie de l'irradiation sur l'importance de l'effet, avec même des possibilités d'inversion d'effet, ainsi que de l'existence de nombreux processus de sélection, intervenant pour des irradiations à niveaux de dose et de débit de dose élevés, permet d'être rassuré sur les conséquences génétiques d'une irradiation supplémentaire de quelques rems par génération.

Il semble qu'il ne soit pas réaliste de calculer et de prévoir des malformations et des morts, à ce niveau-là.

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ESTIMATED RISK FROM ^{239}Pu TO HUMAN BONE, LIVER AND LUNG*

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Abstract

ESTIMATED RISK FROM ^{239}Pu TO HUMAN BONE, LIVER AND LUNG.

The cumulative risks to humans, in terms of average organ doses from ^{239}Pu , are tentatively estimated as follows: bone sarcoma risk = 200 bone sarcomas/ 10^6 person · rad; liver cancer risk = 100 liver cancers/ 10^6 person · rad; and lung cancer risk = 200 lung cancers/ 10^6 person · rad. The risk to bone is based on the sarcoma incidence in German patients receiving long protracted irradiation from repeated injections of 3.62-day ^{224}Ra , an α emitter which, like ^{239}Pu , decays to a large extent on bone surfaces. Independent support for this risk estimate comes from the fact that it exceeds the bone sarcoma risk from long-lived radium in man by roughly the same factor as the risk from ^{239}Pu exceeds that from ^{226}Ra in experimental animals. The risk to liver is based on the observation in beagles injected with low activities of ^{239}Pu that the incidence of liver tumours per rad is about half as great as the incidence of bone sarcomas per rad. The proposed risk to the human liver closely predicts the observed incidence of liver cancer in patients whose livers were irradiated with α -emitting Thorotrast. The risk to lung is based on three factors: the predicted cumulative incidence of all forms of non-leukaemic cancer in the Japanese A-bomb survivors exposed to total body neutron and γ -ray irradiation; the observation that about 1/5 of these radiation-induced cancers were of the lung; and the assumption that α particles and neutrons are about ten times more effective per rad than are high dose-rate γ rays in the induction of cancer. An effort has been made to derive risk estimates that are realistic, rather than too low or too high. However, each of these estimated risks must be regarded as provisional and open to subsequent revision. The proposed risk for lung cancer is especially uncertain, although in rodents the incidence per rad from Pu-induced lung cancers seems somewhat similar to that for Pu-induced bone sarcomas. An inherent complication in each of these estimated risks is to what extent the true dose-responses deviate from the assumed linear relationships. These risk estimates should not be used for organ doses considerably above 1000 rads.

INTRODUCTION

Inhalation is regarded as the most important route of intake of ^{239}Pu into the human body [1]. Inhaled ^{239}Pu moves from the lung to the pulmonary lymph nodes, the skeleton and the liver. Fortunately, the pulmonary lymph nodes are relatively insensitive to plutonium-induced malignancy [1]. Thus, the main organs at risk from ^{239}Pu are the skeleton, the liver and the lung. The purpose of this paper is to estimate numerically the risk to each of these three organs.

I must emphasize that each of these risk estimates is indirect and therefore inexact. Due largely to protective measures restricting the intake of plutonium, no cancer cases definitely attributable to ^{239}Pu have yet been reported in humans. Therefore, the predicted risks to man from ^{239}Pu must be

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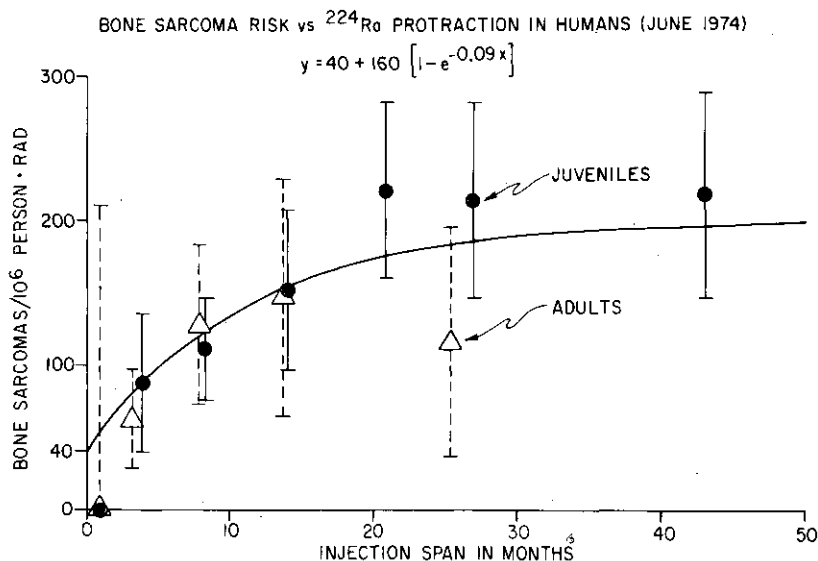


FIG. 1. Bone sarcoma induction versus protraction of ^{224}Ra injections. Data are from Table 2 of Ref. [4] plus one new osteosarcoma in an adult at 728 rads and 11-month protraction. The risk rises to about 200 bone sarcomas/ 10^6 person·rad for long protraction of irradiation from ^{224}Ra . For equal protraction, and with the dosimetric assumptions that have been used [5], juveniles and adults appear roughly similar in bone sarcoma risk from ^{224}Ra .

based on effects produced by other types of radiation in humans and radiobiological evidence from experimental animals. These limitations must be kept in mind continually.

BONE SARCOMA RISK

The estimated risks to bone have been derived in a detailed paper [2] which this section will summarize concisely. ^{239}Pu initially deposits on bone surfaces [3]. With time, part of the ^{239}Pu becomes buried under the apposition of new bone, while part of the ^{239}Pu is liberated by osteoclasts and re-deposited back onto bone surfaces. Thus, some of the ^{239}Pu α particles are emitted from bone surfaces (highly effective) and some from within the bone volume (less effective). The long 24 000-year half-life of ^{239}Pu and its tenacious retention in bone ensure that the irradiation continues for lifetime protraction.

The bone sarcoma risk to man from ^{239}Pu is assumed to be somewhat similar to the risk observed in German patients [4] who received protracted irradiation from repeated injections of ^{224}Ra , an α emitter whose short 3.62-day half-life causes it to decay to a large extent on bone surfaces --- like ^{239}Pu [5]. Figure 1 shows that, for long protraction of ^{224}Ra irradiation, the risk rises to about 200 bone sarcomas/ 10^6 person·rad in terms of average skeletal dose. This estimate predicts that about 200 cases of bone sarcoma may be induced during the lifetime of either (a) 1 000 000 persons each receiving 1 rad average skeletal dose from ^{239}Pu , or (b) 1 000 persons each receiving 1000 rad protracted irradiation from ^{239}Pu , --- but not (c) 1 person receiving 1 000 000 rads since this massive a dose would kill the individual by acute radiation sickness before any cancers could appear. Stated alternatively, the proposed

TABLE I. RISKS IN TERMS OF AVERAGE SKELETAL DOSE (LINEAR MODEL)

Nuclide	C57BL mice		Beagles	Humans
	[Bone sarcomas] [10 ⁶ mouse·rad]		[Bone sarcomas] [10 ⁶ beagle·rad]	[Bone sarcomas] [10 ⁶ person·rad]
	male	female		
²³⁹ Pu	390	1200	5200	200 ^a
²²⁶ Ra	77	70	320	6 to 53
RBE(Pu/Ra)	(5)	(17)	(16)	(4 to 33)

^a Assuming ²³⁹Pu risk = protracted ²²⁴Ra risk. Note that while beagles and mice are more sensitive than humans, the ratio of ²³⁹Pu risk/²²⁶Ra risk seems roughly similar among species.

linear dose-response relationship cannot be valid beyond 5000 rad since an impossible incidence of over 100% would be predicted. The risk estimate is invalid beyond doses of several thousand rad. If the dose response deviates appreciably from the assumed linear relationship at low doses, the risk below 100 rad may require future revision. However, the weight of present experimental evidence with the bone-surface seeking α emitters tends to support an approximately linear non-threshold relationship at low doses [2].

The predicted toxicity to man from bone-surface seeking ²³⁹Pu should exceed that observed in persons, such as dial painters [6], whose skeletons contain long-lived ²²⁶Ra (and ²²⁸Ra) which decay almost entirely within bone volume [7]. Furthermore, one might expect the predicted toxicity ratio of ²³⁹Pu/²²⁶Ra in man to be roughly similar to that observed experimentally in animals. The comparisons shown in Table I suggest that this is indeed the case and provide independent support for the risk estimate of 200 bone sarcomas/10⁶ person·rad for ²³⁹Pu in the human skeleton.

Carcinomas of the head sinuses should not be an important risk from ²³⁹Pu. While they have occurred in the U. S. radium subjects [6], Evans has convincingly ascribed them mainly to the accumulation in the head sinuses of ²²²Rn, the 3.8-day noble gas produced by the decay of ²²⁶Ra, but not by the decay of ²²⁴Ra or ²³⁹Pu [8]. No sinus carcinomas have yet been observed in some 900 German patients injected with ²²⁴Ra and now followed for times up to 29 years after exposure, although 54 bone sarcomas have occurred in this group [4, 5]. Similarly, the risk from ²³⁹Pu-induced leukemia is much lower than that for bone sarcoma [9].

It must be remembered that the proposed risk estimate of 200 bone sarcomas/10⁶ person·rad applies to α emitters, such as ²²⁴Ra, ²³⁹Pu, and ²⁴¹Am, which decay to a large extent on bone surfaces. It does not apply to the long-lived radium isotopes ²²⁶Ra and ²²⁸Ra, which decay almost entirely within bone volume, and it most certainly does not apply to sparsely-ionizing radiations, such as x rays, γ rays, and β particles, which, at low dose-rates, are much less effective than α particles in the induction of malignancy [10-13]. For average skeletal doses below 1000 rad from long-lived ²²⁶Ra and ²²⁸Ra in man, the risk has been estimated to lie between 6 and 53 bone sarcomas/10⁶ person·

rad [2]. For total body γ -ray irradiation the risk to man from the total number of induced fatal malignancies has been estimated on a linear model to be about [25 leukemia deaths + 100 cancer deaths]/ 10^6 person·rad at high dose-rate (above 10 rad/min) or about [5 leukemia deaths + 20 cancer deaths]/ 10^6 person·rad at low dose-rate (below 0.01 rad/min) [10]. On the other hand, it seems likely that the true dose response for sparsely-ionizing radiation may frequently be sigmoid rather than linear [10, 12, 13]. On a dose-squared model (for which the incidence equals the square of the dose times a risk coefficient), the risk to man from total body γ -ray irradiation has been estimated to be about [0.1 leukemia deaths + 0.4 cancer deaths]/ 10^6 person·rad² at high dose-rate (above 10 rad/min) or [0.004 leukemia deaths + 0.016 cancer deaths]/person·rad² at low dose-rate (below 0.01 rad/min) [10].

LIVER CANCER RISK

In our beagles injected intravenously with low activities of ^{239}Pu in citrate solution, the average skeletal dose is approximately equal to the average liver dose, when based on the weights of the organs as irradiated in the living animal (i.e. filled with a normal amount of blood). Since in these beagles (injected with 0.016 and 0.048 $\mu\text{Ci } ^{239}\text{Pu/kg}$) the incidence of liver tumors was half the incidence of bone sarcomas [14, 15], it is tentatively assumed that the risk from ^{239}Pu to the human liver might be half of that to bone, or be about 100 liver cancers/ 10^6 person·rad. It is presumed that the long lifespan in man would allow most of the induced liver "tumors" to become malignant cancers.

The predicted incidence of liver cancer using this assumed risk will now be compared with the observed incidences in patients whose livers were irradiated with α -emitting Thorotrast (colloidal thorium dioxide injected as an x-ray contrast medium). The typical 25 ml of injected Thorotrast gives a liver dose-rate of about 25 rad/year, assuming about 65% of the α energy escapes from the Thorotrast aggregates and is absorbed in tissue [16]. In 20 years the cumulative dose to the liver is (25 rad/year)(20 year) = 500 rad.

$$\begin{aligned} \text{Predicted incidence of liver cancer} &= [500 \text{ rad}] \left[\frac{100 \text{ liver cancers}}{10^6 \text{ person} \cdot \text{rad}} \right] = 5\% \\ \text{Observed incidence in Denmark [17]} &= 32/1012 = 3\% \\ \text{Observed incidence in Portugal [18]} &= 74/1045 = 8\% \\ \text{Observed incidence in Germany [19]} &= 139/1864 = 7\% \end{aligned}$$

While the predicted incidence agrees closely with that observed in the Thorotrast patients, it must be remembered that at least some of the carcinogenic effect on these patients might have been due to chemical irritation [20] from the several grams of thorium in their tissues (25 ml of Thorotrast contains 5 to 6 grams of thorium). Thus, the true risk to the human liver from ^{239}Pu could very well differ from the assumed 100 liver cancers/ 10^6 person·rad.

LUNG CANCER RISK

There are difficulties in estimating the lung cancer risk from ^{239}Pu via comparison with the uranium miners who inhaled ^{222}Rn and its α -emitting decay products. ^{239}Pu in the lung decays mainly within the alveoli (the air sacs deep within the lung), while the highest dose from inhaled ^{222}Rn decay products is received by the bronchial epithelium (the lining of the large air passageways).

In an attempt to bypass some of this difficulty, the risk estimate for ^{239}Pu in the lung will be derived from data on the A-bomb survivors whose

lungs received more or less uniform irradiation from neutrons and γ rays. Therefore, regardless of the location of the critical cells within the lung, one can be sure that these cells were indeed irradiated.

Based on the A-bomb data, the risk from all fatal cancers (excluding leukemia) has been estimated at 100 cancer deaths/ 10^6 person \cdot rem for total body irradiation at high dose-rate assuming a quality factor of about 10 rem/rad for neutrons [10]. But only a fraction of the total cancer deaths are from lung cancers. The BEIR report [21] gives risk rate estimates of 0.60 lung cancers per year/ 10^6 person \cdot rem (p. 152) and 3.0 total cancer deaths per year/ 10^6 person \cdot rem (p. 161). This implies that about $0.6/3.0 = 1/5$ of the induced cancer deaths from total body irradiation are from lung cancer. Perhaps by coincidence, the American Cancer Society [22] estimated that, during 1974, lung cancers accounted for 1/5 of the total cancer deaths in the USA (75 400 lung cancer deaths/355 000 total cancer deaths). Using these values and assuming a quality factor of 10 rem/rad for α particles [23], the estimated risk for ^{239}Pu in the lung is:

$$\left[\frac{100 \text{ cancer deaths}}{10^6 \text{ person} \cdot \text{rem}} \right] \left[\frac{1 \text{ lung cancer}}{5 \text{ cancer deaths}} \right] \left[\frac{10 \text{ rem}}{\text{rad}} \right] = \frac{200 \text{ lung cancers}}{10^6 \text{ person} \cdot \text{rad}}$$

Complications exist. This risk estimate was derived from a mixed population of smokers and non-smokers. Cigarette smoking may increase the risk of ^{239}Pu irradiation to an individual while non-smoking may decrease it. It is unknown whether the concentration of "critical cells" capable of being transformed into cancer by irradiation in the alveolar region corresponds to that averaged over the total lung. ^{239}Pu irradiates the lung as discrete "hot particles", whereas the risk estimate has been derived from uniform irradiation of the total lung. However, the available scientific evidence indicates that "hot particles" in the lung are probably not more carcinogenic than the same retained activity more uniformly distributed, and that the hot particles may well be less hazardous [24]. Thus, the true risk to the average population could be considerably different than estimated. Certainly the estimated risk for the lung seems less certain than the other risk estimates in this paper.

However, the numerical equality between the proposed risk estimates for bone and lung could be more than coincidence. Thompson has noted a striking similarity between the observed incidences of bone sarcomas and lung cancers at equal average organ doses in rodents [25] (compare his Figs 1 and 2). There is no question that better estimates of the ^{239}Pu risk to the human lung are urgently needed.

PREDICTED EFFECTS FROM PRESENTLY "PERMISSIBLE" ORGAN BURDENS OF ^{239}Pu

The cancer incidences will now be estimated which might result if the skeleton, liver and lung were continually maintained at their presently maximum permissible dose-rates from ^{239}Pu for an occupational work time of 50 years.

The skeleton of an occupationally exposed worker is permitted 30 rem/year. Using a quality factor of 10 for α particles and a distribution factor of 5 for ^{239}Pu in bone, the permissible dose-rate is $30/(10 \times 5) = 0.6$ rad/year, which gives a 50-year dose of 30 rads. The liver and lung are permitted 15 rem/yr, which with a quality factor of 10 and a distribution factor of 1 for soft tissue corresponds to 1.5 rad/year or 75 rad in 50 years. The corresponding permissible organ burdens are 0.044 μCi (skeleton), 0.028 μCi (liver), and 0.016

μCi (lung). Using the risk estimates derived in this paper, the predicted cancer incidences are:

$$\text{Skeleton} = [30 \text{ rad}] \left[\frac{200 \text{ bone sarcomas}}{10^6 \text{ person} \cdot \text{rad}} \right] = 0.6\%$$

$$\text{Liver} = [75 \text{ rad}] \left[\frac{100 \text{ liver cancers}}{10^6 \text{ person} \cdot \text{rad}} \right] = 0.8\%$$

$$\text{Lung} = [75 \text{ rad}] \left[\frac{200 \text{ lung cancers}}{10^6 \text{ person} \cdot \text{rad}} \right] = \underline{\underline{1.5\%}}$$

Total incidence of ^{239}Pu -induced cancers $\approx 3\%$

In other words, the lifetime maintenance of a presently permissible ^{239}Pu organ burden in one of these organs might convey roughly a 1% chance of radiation-induced cancer, but roughly a 3% chance if all three were kept at their permissible organ burdens.

For comparison, the permissible body burden of $0.1 \mu\text{Ci } ^{226}\text{Ra}$ (assuming nearly all is in bone) gives an average skeletal dose-rate of 3 rad/year and a cumulative dose of 150 rad in 50 years. Assuming the bone sarcoma risk is between 6 and 53 bone sarcomas/ 10^6 person·rad from ^{226}Ra (Table I), the total risk from ^{226}Ra should lie between about 9 and 80 total cancers/ 10^6 person·rad, since ^{226}Ra induces about 1 carcinoma of the head sinuses for every 2 induced bone sarcomas [6]. The total predicted induction of cancer from a maintained permissible occupational body burden of $0.1 \mu\text{Ci } ^{226}\text{Ra}$ would then be: (150 rad)(9 - 80 total cancers/ 10^6 person·rad) = 0.14% to 1.2%.

This overlaps the 0.6% incidence of bone sarcomas predicted for the permissible skeletal burden of ^{239}Pu , but is less than the predicted 3% incidence of all induced cancers from the simultaneously maintained permissible organ burdens of $0.044 \mu\text{Ci } ^{239}\text{Pu}$ in the skeleton, $0.028 \mu\text{Ci}$ in the liver, and $0.016 \mu\text{Ci}$ in the lung. Therefore, although the presently permissible body burden of $0.04 \mu\text{Ci}$ of ^{239}Pu was based on the incorrect assumption that nearly all of the body burden was in the skeleton, it may be appropriate to restrict the total body burden to $0.04 \mu\text{Ci } ^{239}\text{Pu}$, or perhaps a somewhat lower value, so that the total risk will be (a) similar to that from $0.1 \mu\text{Ci } ^{226}\text{Ra}$, and (b) similar to the risks in other occupations which are considered to be acceptably safe.

PREDICTED EFFECTS FROM INHALATION OF $1 \mu\text{Ci } ^{239}\text{Pu}$

The ICRP Report on the metabolism of plutonium [26] contains a retention model from which one may calculate that, for the inhalation of $1 \mu\text{Ci}$ of ^{239}Pu in particulates with an activity median aerodynamic diameter of about $1 \mu\text{m}$, the resulting doses to "reference man" 50 years later would be about 13 rad to the 7000-g skeleton, 40 rad to the 1800-g liver and 30 rad to the 1000-g lung. The actual doses likely differ somewhat from these calculated values and are influenced by the in vivo solubility characteristics of the inhaled particulates, which depend on chemical form, physical size, and concentration of radioactivity. However, using the calculated doses as best present estimates, the predicted cancer incidences per inhaled μCi of ^{239}Pu are:

$$\text{Skeleton} = [13 \text{ rad}] \left[\frac{200 \text{ bone sarcomas}}{10^6 \text{ person} \cdot \text{rad}} \right] = 0.3\%$$

$$\text{Liver} = [40 \text{ rad}] \left[\frac{100 \text{ liver cancers}}{10^6 \text{ person} \cdot \text{rad}} \right] = 0.4\%$$

$$\text{Lung} = [30 \text{ rad}] \left[\frac{200 \text{ lung cancers}}{10^6 \text{ person} \cdot \text{rad}} \right] = \underline{\underline{0.6\%}}$$

Total incidence of ^{239}Pu -induced cancers $\approx 1\%$

It may be convenient for planning purposes to assume tentatively that if 100 persons of mixed ages each inhaled 1 μCi of ^{239}Pu , one of these persons might die from plutonium-induced cancer.

CONCLUSION

While these risk estimates are subject to considerable uncertainty, the predictions emphasize the continued advisability of keeping plutonium exposures as low as practicable.

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DISCUSSION

P.G. GROER: The use of ^{224}Ra for ^{239}Pu risk estimates is an ingenious idea. But one fact bothers me. The bone tumours for the ^{224}Ra cases occur long after the last ^{224}Ra atom has decayed. Those due to exposure to ^{239}Pu , however, seem to appear while the tissue at risk is still being irradiated. From the standpoint of radiation biology, this represents a completely different situation.

C.W. MAYS: For short periods of irradiation, the distribution of appearance times for ^{224}Ra -induced bone sarcomas is quite similar to that observed for induced leukaemias in the Japanese A-bomb survivors (O.J. Bizzozero et al., New Engl. J. Med. 274 (1966) 1095). In the German patients, the earliest induced bone sarcoma appeared 3.5 years after the initial ^{224}Ra injection. The sarcoma frequency peaked at 6 to 8 years, but then greatly declined so that by 20 years the majority of bone sarcomas seem to have been expressed. In fact, the average latent period is only about 10 years, suggesting that for continuous irradiation from ^{239}Pu during a 50-year occupational work time, most of the induced bone sarcomas would have time to appear before death. Only during the last few years of life would ^{239}Pu irradiation be incapable of producing an observable bone sarcoma.

M. DELPLA: You have based your arguments on linear relations, especially on a risk of $100/10^6$ man·rem for death from lung cancer. Studying the variation of this risk with dose, at Nagasaki, we find a

variation with negative concavity, i.e. a negative added risk. It would be dangerous, on the basis of such non-significant results, to state that doses of 100 rads or less have a protective effect.

But an assumption to the contrary is also dangerous in that it would frighten the public.

C.W. MAYS: Indeed, at Nagasaki, where nearly the whole dose was from low-LET γ rays, the dose-response for leukaemia appears highly sigmoid. But the dose-response was linear at Hiroshima, where more than twice as many persons were exposed and the radiobiological damage was primarily from high-LET radiation (neutrons). My ^{239}Pu risk estimates are also for high-LET radiation (α particles). Judging by the preponderance of present evidence on the dose-response for densely ionizing radiations, particularly for those α emitters which are deposited on bone surfaces, I cannot ignore the strong possibility that at low doses from ^{239}Pu the incidence of cancer increases somewhat linearly with dose.

With regard to the question of frightening people, I don't think that you need to apologize at all because the risks from nuclear power are much lower than those from burning coal. The few cancers that might result from nuclear power are insignificant in comparison with the much larger health damage from the coal required to produce the same amount of power. You are to be congratulated on your efforts to provide clean nuclear power for France.

M. DELPLA: Do not talk about coal-fired power plants, Mr. Mays, please. The public will not compare nuclear power plants with coal-fired plants, nor natural radioactivity with the activity from nuclear plants. If natural radioactivity gives 100 cancers, they do not want the 101st cancer that is attributed to a nuclear plant.

C.W. MAYS: I understand the way you feel emotionally about this question, but I can only evaluate it on the basis of the scientific evidence. For low-LET radiation from X rays, γ rays and β particles, the dose response is often sigmoid due to greater opportunity for repair at low doses and low dose rates. I myself have written papers on the sigmoid dose responses for leukaemia induction in persons at Nagasaki by A-bomb γ rays [10], the induction of bone sarcomas in mice, rats and beagles by ^{90}Sr β particles [12] and the induction of thyroid tumours in children by ^{131}I β particles [13]. Originally, I had been led to expect a sigmoid dose-response for α irradiation and, indeed, this is sometimes seen in the case of the long-lived isotopes of radium which decay largely within bone volume. Occasionally, the response is concave downwards with a greater effect per rad at the lowest dose, such as Arne Luz has reported at this symposium (paper SM-202/406) for bone sarcomas induced in mice by single injections of ^{224}Ra . But the most commonly observed dose-response at low doses from α particles seems approximately linear for bone sarcoma induction by ^{224}Ra in humans, ^{239}Pu and ^{228}Th in beagles, ^{239}Pu in rats and ^{226}Ra , ^{227}Th and ^{239}Pu in mice [2].

A recent and appealing theory of radiation-induced cancer (K.H. Chadwick and H.P. Leenhouts, IRPA Congress at Washington, D.C., CONF-730907-P1 (1974) 457) holds that a double-strand break in DNA is usually not repaired and may lead to cancer, whereas a single-strand break can usually be repaired and thus rarely leads to cancer, providing that a nearby break in the other strand does not occur before the first break has been repaired.

Since a single α particle can break both strands simultaneously it would appear that the dose-response for α irradiation may have a linear component even at very low doses. Theoretical considerations by Harald Rossi and Albrecht Kellerer lead to the same conclusion.

On the basis of present evidence it would be very difficult for me to conclude that there should be zero effect from low doses of α irradiation, however fervently one might wish for this to be the case. We cannot legislate the laws of radiobiology.

H.H. ROSSI (Co-chairman): Theoretical considerations clearly indicate that there must be a linear relation between the absorbed dose of any radiation and cellular effects. However, the response of organized tissues cannot at present be predicted theoretically.

C.W. MAYS: I certainly agree with Dr. Rossi that theory is no substitute for the actual experimental data.

R. WILSON: Dr. K.Z. Morgan (Am. Ind. Hyg. Assoc. J., August 1975, p.567) has recently proposed a reduction in the maximum permissible bone burden of ^{239}Pu by a factor of 240. On the basis of your risk estimate of 3%, this would mean a very low risk of (3/240)%. Would you like to comment?

C.W. MAYS: I am familiar with Dr. Morgan's paper. He started with the maximum permissible body burden of $0.04 \mu\text{Ci}$ for ^{239}Pu , which was based on the assumption that the skeleton was the critical organ and that nearly all the retained plutonium was in bone. He then applied a series of four correction factors which, when multiplied together, suggested to him that the present body burden should be reduced by a factor of 240.

His first reduction factor of 3 assumes that the relative damage factor N for ^{239}Pu in bone should be 15, on the basis of our beagle results, rather than the N factor of 5 assumed at present. The effect of this change would reduce the body burden by a factor of $15/5$, or 3. Indeed, our latest results indicate that ^{239}Pu is about 16 times more toxic than ^{226}Ra (on the basis of our chemist's latest revisions of average skeletal dose for ^{239}Pu), so I am in full agreement with Dr. Morgan on this point.

His second reduction factor of 2 is based on the work of Elizabeth Lloyd and D. Hodges (Clin. Orthop. 78 (1971) 230), which indicated that the surface-to-volume ratio for the trabecular bone of beagles is about twice that for man. Thus, the same amount of ^{239}Pu per cm^3 of bone would have twice the surface concentration in man as in dogs. Dr. Morgan proposed a reduction factor of 2 in the light of this consideration. This approach seems reasonable to me, although "in press" results of F.W. Spiers and Joan R. Whitwell ("Dosimetry of ^{239}Pu and ^{226}Ra in man and animals", in Biological Effects of Plutonium and Radium (W.S.S. Jee, Ed.), J.W. Press, Salt Lake City, Utah) suggest the possibility that the surface-to-volume ratio may be rather similar in beagle and human trabecular bone, and that the corresponding reduction factor may therefore be about 1. Perhaps an average reduction factor of about 1.5 might be appropriate.

Dr. Morgan's third reduction factor of 10 is based on the assumption that the rate of burial of ^{239}Pu by the apposition of new bone is ten times faster in the dog than in man. But man lives five times longer than the dog and therefore has five-fold more time in which the burial processes can take place. Therefore I would consider the appropriate reduction factor to be about $10/5 = 2$. Using a complex mathematical model, and

assuming average latent periods for bone sarcomas of 30 years in man and 10 years in the dog, John Marshall and Elizabeth Lloyd (in Radionuclide Carcinogenesis, AEC Symposium Series 29, CONF-720505 (1973) 421) have derived a factor of 3.

Dr. Morgan's fourth reduction factor of 4 is based on the suggestion that the baboon lung is about four times as radiosensitive to ^{239}Pu as the dog lung, and that the same ratio would apply to human bone versus dog bone. However, I am afraid that an error in logic has been made here since the comparison equation:

$$\frac{{}^{239}\text{Pu toxicity in bone}}{{}^{226}\text{Ra toxicity in bone}}\bigg)_{\text{man}} = \frac{{}^{239}\text{Pu toxicity in bone}}{{}^{226}\text{Ra toxicity in bone}}\bigg)_{\text{dog}}$$

does not assume equal toxicities of Pu in man and in the dog but only an approximate equality in the ratio of $^{239}\text{Pu}/^{226}\text{Ra}$ toxicities. Actually, as can be seen from my Table I, man is about 26 times less (not more) sensitive than the beagle per rad of average skeletal dose from bone-surface seekers. But man is also less sensitive than the beagle (by roughly the same factor) per rad of average skeletal dose from the bone-volume seekers, so the ratio of toxicities appears to be roughly the same among species. Dr. Morgan himself has pointed out that his fourth reduction factor may have been based on an incorrect assumption, and indeed this appears to be the case.

In summary, our evaluation of the total reduction factor would be:

Evaluation by Dr. Morgan = $(3)(2)(10)(4) = 240$

Evaluation by myself = $(3)(1.5)(2)(1) = 9$

While some reduction in the maximum permissible bone burden of ^{239}Pu may be appropriate, it seems to me that a factor of 240 is overdoing it somewhat. On the basis of other considerations, even a factor of 9 might be excessive.

Y. NISHIWAKI: I think this type of information on comparative estimates of risks would be extremely important for hazard evaluation of some of the new types of reactors such as fast breeders. However, measurements of significant amounts of plutonium isotopes in the environment have demonstrated that ^{238}Pu , ^{240}Pu , ^{241}Pu , ^{241}Am , etc., in addition to ^{239}Pu , are already present in small but detectable amounts. There are some reports indicating that the behaviour of ^{241}Am introduced into the environment as americium appears to be different from that of ^{241}Am produced by β decay of ^{241}Pu with a half-life of about 13.2 years in the environment, possibly because of the "in situ" generation of ^{241}Am from ^{241}Pu at the point of deposition of plutonium. I think it would be extremely important, if these studies are continued, to include comparative estimates of the different isotopes of transuranics which may be introduced into our environment in the future.

C.W. MAYES: We began the study of ^{241}Am in beagles about nine years ago. It appears, tentatively, that the risk per rad to the skeleton from ^{241}Am is similar to that from ^{239}Pu , but the distribution within the body is different. ^{241}Am injected into the bloodstream goes more to the liver but less to the skeleton than ^{239}Pu . The work from other laboratories indicates

that inhaled ^{241}Am moves somewhat more rapidly than ^{239}Pu from the lung into the rest of the body. This means that, following the inhalation of ^{241}Am , the induction of lung cancer may be somewhat less than that from the same inhaled activity of ^{239}Pu . However, the translocating ^{241}Am should produce more liver cancers but fewer bone sarcomas than ^{239}Pu , so that the total risk per inhaled μCi may be somewhat similar. I agree that the retention and toxicity of the important transuranics must be studied. In addition to americium, studies of curium and californium are also in progress in our laboratory.

CARCINOGENIC AND GENETIC HAZARD FROM BACKGROUND RADIATION

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Abstract

CARCINOGENIC AND GENETIC HAZARD FROM BACKGROUND RADIATION.

Various models and predictions of the carcinogenic and genetic hazards of low-level, low-rate ionizing radiation were examined and compared with actual experience in population in the United States of America and elsewhere. All the models predicted a significant increment in malignant mortality, and mortality from genetic disorders, with increasing background. Populations were first examined for malignant mortality, for various age and geographic groups, for all malignancies (ICD 140-205). They were then re-examined for groups of malignancies (e.g. 140-159, 160-164, etc.), and for each of 56 separate malignancy types. These groups and their malignancy rates were compared with the radiation background characteristic of each geographic group. Simultaneous regressions were also performed against over 40 other geographical, social, medical, meteorological, economic, educational, ethnic and pollution parameters. Observation of the populations at risk showed not only no increment in malignant mortality with increasing background, but a consistent and continuous decrement. Similar results were obtained for mortality from congenital malformations. The question of whether low-level radiation, at rates of the order of 0.5 rem/a and below, constitutes a significant environmental hazard is discussed.

1. INTRODUCTION

In recent years the hypothesis has been advanced that a significant fraction of human cancer mortality may be due to the radiation background [1-9]. Although a number of different models and estimations have been advanced [1-9], they all agree in predicting an increase in malignant mortality with increasing irradiation. For each increment of 170 mrem/yr their authors have estimated U. S. cancer mortality excesses of about 3000 to 100 000 per year, i.e. about 1% to 30% of current U. S. experience.

2. ANALYSIS: MALIGNANT MORTALITY

To test these models, plots of the U. S. age-adjusted, malignant mortality rates (r) [10-12] were made against natural backgrounds [13] for the 50 states [14]. These showed, if anything, the reverse (e.g. Fig. 1). Of the 14 states at backgrounds above 140 mrem/yr, 12 were very significantly ($P < 0.01$) below the U. S. average, one insignificantly lower, and only one slightly higher [13]. The probability of this occurring by pure chance proved to be < 0.001 . Conversely, the 10 states in the U. S. with the lowest rates all lay at backgrounds ≥ 135 mrem/yr. Similar results were obtained using other estimates for the natural backgrounds of these state populations [15-17]. Thus, there seemed to be some real, if hidden, association between high backgrounds and low malignant mortalities. A similar, and even more dramatic, effect was noted in the non-white population. However, we confined ourselves

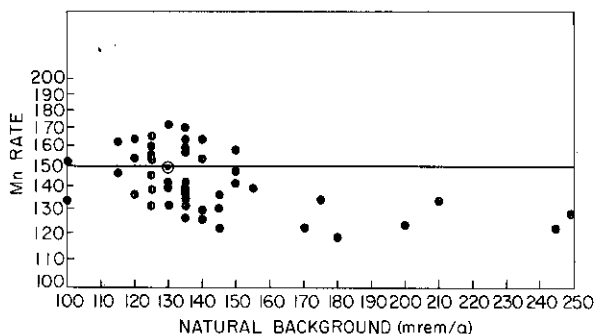


FIG. 1. Age-adjusted malignant mortality rates for the population of the United States of America, 1950-1967, by State and background. The horizontal line and open circle indicate the rate and background for the USA as a whole. Some States at common background have rates identical to the third significant figure, so that a few points actually represent pairs. Each point represents about 10^5 deaths, on the average, so that statistical errors are less than the size of the points.

to the white population in this study because of its greater homogeneity, better statistics, the better availability of socioeconomic data, etc.

For purposes of further comparison, we discriminated three groups: A, the seven states of natural background above 165 mrem/yr; B, the fourteen states of natural background above 140 mrem/yr; C, the fourteen states with the lowest backgrounds. These were compared with all 50 U. S. states, and some of the more pertinent results are summarized in Table I.

We first analysed the states by malignancy (Mn) type [13], using the coding system of the International Classification of Deaths (ICD) [13], to see if the low mortalities of groups A and B could be due to particularly low rates for a few Mn types. These two groups, however, proved to be lower than the U. S. as a whole for all of the 56 Mn types tested, and this premise had to be discarded. A summary is presented in lines 3-7 of Table I, with the 56 types grouped into common categories (e.g. 140-159 Oral, Digestive and Peritoneum; 160-164 Respiratory; 200-205 Hematopoietic). The rates for all categories, in fact, tended to decrease with increasing radiation background.

The rates for the seven malignancies which have been cited [7] as most likely to reflect radiation carcinogenesis are displayed separately in lines 8-14. With one exception (the leukemia rate of group B), the rates for groups A and B are noticeably lower than for the U. S. as a whole and, again, the trend is for rates to decrease as radiation backgrounds increase. It has recently been shown that leukemia rates tend to increase slightly with altitude up to about 2000 feet (approximately the mean altitude of group B), but then to decline with further increases in altitude [18,19]. However, state-by-state study of leukemia rates showed no correlation with radiation backgrounds, even for group B [20].

The models so far proposed have also held that the greatly increased radiosensitivity of the young should lead to a marked increase in malignant mortality, relative to adults, for the same radiation background [1-9, 21]. In fact, as shown in lines 15 and 16, the reverse is observed. The age-specific rates (R) among the young of groups A and B are decreased relative to those of C and/or U. S., and in much the same ratios as the age-adjusted malignant mortality rates.

TABLE I. U.S. LOW AND HIGH BACKGROUND, WHITE POPULATIONS, 1950-1967

No.	Characteristic	A	B	U. S.	C
1	Natural background, mrem/yr	210	170	130	118
2	White population, thousands	5 735	16 897	158 051	59 683
3	r, Mn 140-159	42.9	45.6	52.4	50.3
4	r, Mn 160-164	15.8	16.9	22.3	23.4
5	r, Mn 170-181	36.8	38.2	41.5	40.1
6	r, Mn 190-205	30.8	31.5	33.3	33.0
7	r, All malignancies	126.3	132.2	149.5	146.8
8	r, Stomach, 151	11.7	11.6	11.8	11.0
9	r, All G. I., 150-159	40.7	43.0	49.0	46.7
10	r, Lung, 163-164	14.5	15.5	20.4	21.5
11	r, Breast, female, 170	21.5	22.6	25.3	24.4
12	r, Thyroid, 194	0.055	0.054	0.057	0.054
13	r, Bone, 196	0.92	1.03	1.12	1.07
14	r, Leukemia, 204	7.03	7.23	7.13	6.91
15	R, Mn 140-205, age 0-9	8.11	8.31	8.54	8.31
16	R, Mn 140-205, age 10-19	6.80	6.61	6.82	6.72
17	R, Mn 140-205, age 20-29	10.46	10.73	11.09	11.19
18	R, Mn 140-205, age 30-39	27.61	28.39	31.45	32.27
19	Mortality rate, all causes	892.0	893.2	928.5	903.9
20	U. S.-group, all causes	36.5	35.2	-	24.6
21	U. S.-group, malignancy	23.2	17.3	-	2.7
22	Mortality fraction, 740-59	0.152	0.159	0.177	0.180
23	Mortality fraction, fetal	0.56	0.58	0.60	0.61

Even if the low spontaneous rates of the young, combined with the predominantly embryogenic character of childhood malignancy, were to somehow mask the radiation effect in youth, an increase should nonetheless be evident by middle age [1-9]. Again, as shown in lines 17 and 18, the age-specific rates for young adults, and those in early middle-age, are lower for groups A and B, relative to U. S. and C, rather than higher.

Certain possible explanations for this pattern were ruled out by the nature of the observed pattern for total mortality (line 19). If the decedent populations of A or B were to contain significant numbers of immigrants from other parts of the U. S., i.e. decedents who had not been exposed to the high radiation backgrounds of A and B until late in life, one would anticipate an increase in the Mn rates of A and B on this basis alone. This arises because the Mn rates of the remaining states are much higher than those for A or B alone, e.g. 146.8 for C, 150.4 for the U. S. -- minus -- A, and 151.6 for the U. S. -- minus -- B. Instead, the reverse was observed. Thus, if short-term residents are a significant factor, the true rates for long-term residents must be even lower than those shown in Table I.

The problem of competing risks was then examined, i.e. are the populations of A and B dying of some other cause, so that their members are removed before malignancy can become manifest? Age-adjusted rates for total mortality (line 19) were used to obtain mortality decrements for all causes (line 20), and for malignancy alone (line 21). All three of the groups show lower total mortalities than the U. S. as a whole, but A and B are the lowest of the four. The malignant decrement, however, (e.g. U. S. -- minus -- A, etc.) increases rapidly with increasing background. Indeed, in the very highest background group, A, the malignancy decrement is very nearly equal to the total decrement. This is just the reverse of a case of competing risks, at least in the sense given above.

3. ANALYSIS: GENETIC EFFECTS

Although the models cited are not all as clear on the probable genetic effects of background radiation as they are on carcinogenic effects, they do all agree in predicting an increase in serious effects with increasing irradiation [1-3, 6, 7, 21-24]. For each increment of 170 mrem/yr these models have estimated increments of serious deleterious genetic effects of the order of 10^2 to 10^5 per year. Since congenital malformations were usually taken to be an important component of these effects, we examined the U. S. mortality patterns from congenital malformations, ICD 740-759 [11, 12]. We felt that, even if the rates themselves might be too subject to local factors to provide reliable indications of effect [22-24], at least the ratio of congenital defect mortality to total mortality might be expected to reflect increases due to increased irradiation. These ratios are shown, in line 22 of Table I, for the first year of life.

Again, even if radiation effects did not show up as congenital malformation mortality, perhaps it would show up as an increase in fetal mortality, e.g. via an increase in dominant lethals [1-3, 6, 7, 21-24]. The ratios of fetal mortality to total first-year mortality is shown in line 23 of Table I. In each case the values obtained decreased with increasing background radiation, much as did the malignant mortalities. Thus, we were unable to confirm any positive effect of increasing background radiation on deleterious genetic effects.

TABLE II. CHARACTERISTICS OF POPULATION GROUPS

No.	Characteristic	A	B	U. S.	C
1	Cosmic ray dose, mrem/yr	105	72	45	42
2	Terrestrial dose, mrem/yr	80	72	60	51
3	Mean altitude	5400	2900	2500	1300
4	Residence altitude, ft.	4510	2650	900	730
5	Annual mean temp., °F	50	51	55	59
6	Annual precip., inches	14	25	35	36
7	Days/yr with precip.	86	97	115	99
8	% of possible sunshine	69	65	60	63
9	Urbanization, %	63	57	69	74
10	Per capita personal income, \$	2021	1922	2215	2255
11	Median family income, \$	5600	5400	5660	5650
12	Physicians/1000 population	1.27	1.25	1.49	1.49
13	Hospital beds/1000 population	8.24	8.82	9.49	8.76
14	Median years of school completed	11.8	11.7	10.9	10.8
15	Poor diet households, %	16.5	21.2	19.1	19.1
16	Population on Federal Food Assist., %	2.6	3.2	3.2	2.5
17	Unemployment, %	4.3	3.9	3.9	3.3
18	Accepted, Military Selective Service, %	65	63	56	53
19	Life expectancy, male	67.7	67.7	67.6	67.5
20	Life expectancy, female	74.5	74.7	74.2	74.3
21	Urban air, particulates, $\mu\text{g}/\text{m}^3$	129	119	115	116
22	Urban air, benzene soluble, $\mu\text{g}/\text{m}^3$	10.1	9.3	9.5	9.6
23	Urban air, radioactivity, pCi/ m^3	8.5	7.7	6.8	6.3
24	Urban air, beta, pCi/ m^3	5.5	5.2	4.4	4.2

4. CORRELATIONS

In an attempt to find some secondary association, regressions were run against background, and against rate, for some 40 factors [12, 14]. These included: geographical factors (altitude, temperature, rainfall, etc.); demographic factors (ethnic makeup, life expectancy, urbanization, migration, population growth, etc.); physical factors (medical radiographic exposures, atmospheric pollution, fallout levels, etc.); and socioeconomic factors (personal and family incomes, schooling, unemployment, crime rates, medical facilities, dietary levels, etc.). Few associations were found beyond the obvious ones, i.e. high backgrounds in the U. S. tend to be associated with higher altitudes because of the increased cosmic-ray component. Thus, groups A and B tended to be cooler, drier, and at higher altitudes than C or the U. S. The few correlations found outside these geographic ones were in a direction inverse to that which would be expected from the low malignant mortality rates of A and B, e.g. groups A and B had higher fallout and pollution levels, lower personal and family incomes, more unemployment, and longer lives than either C, or the U. S. A summary of some of the more pertinent values is given in Table II. We were unable to find any correlation which would provide a reasonable secondary association for the observed background and mortality rates.

5. CONCLUSION

The models referred to above have all derived their data from small-population studies, at high dose-rates, and generally at high dose levels. They have assumed that the dose-response curves so obtained extrapolate monotonically to zero, show no threshold of response, and are independent of dose rate [1-9, 21-26]. And it is on these bases that predictions have been made of significant increments in malignant and genetic mortality for radiation levels less than or equal to those of the natural background.

Observation of the actual populations at risk shows not only no increment, but an actual decrement, so that these predictions are left quite without observational support. It appears that one or more of the above assumptions is probably invalid, as has been suggested [25, 26], and that low-level, low-rate radiation probably does not constitute an environmental hazard of significance.

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DISCUSSION

C.W. MAYS: The mountain States of high background radiation are also States where a significant fraction of the population are Mormons, members of a group which strongly disapproves of smoking and encourages clean living. In Utah, the incidence of lung cancer is only half the national average. Think about that as you stamp out your cigarette!

N.A. FRIGERIO: Frankly, as a clergyman myself, I hope that clean living is the reason for the lower rates.

R.B. HOLTZMAN: Could the variations found in your study be due to insufficient data on the radiation dose? The tables seem to give only the external radiation levels but not the levels of internal dose, probably because they are poorly known and cannot be easily measured by flying an aircraft over the countryside. An example of high internal radiation with low external levels can be found in the parts of Illinois which have high ^{226}Ra levels in municipal water supplies. People drinking these waters have much higher skeletal radiation levels than the general population.

N.A. FRIGERIO: Table I does include internal background, so far as we knew it. We are currently re-examining internal backgrounds in the populations studied, and our results so far show considerably higher internal backgrounds in groups A and B than in the USA or group C.

The reason is that these populations live not only at higher altitude but also in regions having high soil and water radioactivity and high atmospheric radon content.

R.B. HOLTZMAN: If radiation-induced cancer from low levels is of marginal significance, another factor in play is that based on military acceptance rates - the people in the high-radiation areas appear to be generally healthier and thus, from the standpoint of general health, the populations in the high- and low-radiation areas on the whole are different. This matter of health could be important if there is a correlation between general health and incidence of malignancy.

N.A. FRIGERIO: The high-radiation area dwellers are certainly healthier. If Dr. Mays is right, they are enjoying the benefits of clean living.

D.S. WOODHEAD: Are the areas of high background radiation generally situated at high altitude?

N.A. FRIGERIO: Generally, but not always.

D.S. WOODHEAD: In that case, have you looked at the possible influence of an altered oxygen tension on cancer incidence, since a reduced oxygen tension might be expected to reduce the radiation effect?

N.A. FRIGERIO: Yes, we have, but from none of the models and data on oxygen effect could we find anything which would explain these results. Complete lack of oxygen has a reduction factor of ≤ 3 ; so even in that case predicted increases would only have changed from $+3 - 30\%$ to $+1 - 10\%$.

W.K. SINCLAIR: What is the standard deviation which you would expect in the incidence numbers in order to be sure that the correlation is positive?

N.A. FRIGERIO: In almost all cases the standard deviation was only about 0.01.

W.K. SINCLAIR: If you assume the linear hypothesis, how much should this increase the incidence between 130 mrem and 210 mrem? My rough estimate is about 0.1, and I don't see how you could expect to see this.

N.A. FRIGERIO: With standard deviations smaller than 0.01, we felt that we should see the predicted increases of, say, ≥ 0.1 . Then, too, we actually got negative changes. So we were looking at decrements ≥ 20 times the standard deviations.

P.G. GROER: Since you mentioned the problem of competing risks, I take it that the risks reported in your paper are "net". Did you assume that the different risks act independently in your derivation of these "net risks"?

N.A. FRIGERIO: At all events we did not separate risks. We just took their differences, as shown in Table I.

R.J.M. FRY: How do you eliminate the possibility of the quality of certification being lower in areas of high background or high altitude than in those areas with low background, especially if high background areas are also areas of lower income?

N.A. FRIGERIO: We did not actually find that. For example, quality in Denver seemed to be at least as good as elsewhere. It is perhaps for this reason that the National Cancer Institute uses Colorado in its incidence studies. But, of course, the quality of the data is no better than that of the United States vital statistics as a whole.

LOW-DOSE IONIZING RADIATION: CARCINOGENIC EFFECTS IN MAN

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Abstract

LOW-DOSE IONIZING RADIATION: CARCINOGENIC EFFECTS IN MAN.

From epidemiologic studies, carcinogenic effects associated with radiation doses under 50 rads are presented, dose-effect relationships are considered, and potentials for making further observations in human populations are reviewed. Malignant lesions reported to be associated with doses of less than 50 rads are leukaemia and thyroid cancer; dose-effect information for these neoplasms is presented. Some studies suggest there may be low-dose associations with several other types of malignancies. The studies reviewed had periods of observation of 16-29 years post-exposure. It is important that studies which are capable of providing data about low-dose effects or dose-response relationships ranging from high to low doses be carried out for as long a period of follow-up as possible.

Although there have been many studies of radiation carcinogenesis in human populations, few have dealt with the consequences of low-level exposures. The unsatisfactory state of extrapolation from high doses [1,2], and the increasing importance of assessing low-dose effects as a basis for radiation protection standards, make it worthwhile to examine available reports of low-level radiation carcinogenesis in man. To this end, we have assembled reports of carcinogenic effects associated with doses of less than 50 rads in various follow-up studies, looked for dose-response relationships, and noted potentials for additional observational data in humans.

MALIGNANT NEOPLASMS AND DOSES UNDER 50 RADS

The malignant lesions found associated with estimated doses of less than 50 rads in epidemiologic studies of human populations are leukaemia and thyroid cancer. In addition there is suggestive or indirect information about induction of cancer of the parotid gland, skin, and solid tumours of childhood.

An excess of low-dose radiation-induced leukaemia has been observed in the survivors, all ages, of the atomic bomb explosion in Hiroshima who were exposed to 20 - 49 rads, as reported by Ishimaru et al. [3]. An increased risk of leukaemia has also been observed in children who were exposed in utero to diagnostic X rays in the dose range of 0.5 - 2.0 rads, investigated by Stewart and colleagues in the United Kingdom [4], and by MacMahon [5] and Diamond et al. [6] in the United States of America. A thyroid dose of 6.5 rads in children who received X-ray epilation therapy for tinea capitis years ago in Israel has been associated by Modan et al. [7] with increased risk of thyroid cancer.

A significant excess of parotid gland cancer, as well as thyroid cancer, was found in the irradiated group in the tinea capitis study in Israel. An estimate of the radiation dose to the parotid gland is not yet available from Israel; however, a parotid dose of 39 rads has been determined by Harley and others in New York City, where a similar but smaller tinea capitis irradiation study is being conducted [8]. The latter study has found only one of the excess salivary gland neoplasms to be malignant to date, as reported by Shore et al. [9]. The tinea capitis study in New York City also provides some information about cancer of the skin. White children who received scalp X irradiation two to three decades ago have been found to have excess basal cell carcinomas of the head and neck region. Of eleven such tumours in the X-rayed group, four have developed in shielded areas (nose, around the eyes, and anterior neck) where skin radiation doses were estimated to be 20-45 rads. Attention was drawn to the high frequency of basal cell cancers in irradiated parts of the head exposed to solar u.v. radiation [9]. Finally, solid malignant tumours of various types have been reported by Stewart and colleagues [4,10] to occur in excess in children exposed pre-natally to 0.5-2.0 rads of diagnostic radiation.

Of the study groups described, the Hiroshima population received a single exposure to radiation that was mixed γ and neutron radiation. The other groups, irradiated during prenatal, infant and childhood periods, had one or more exposures to diagnostic or therapeutic X rays. Follow-up periods ranged from 16 to 29 years.

DOSE-RESPONSE RELATIONSHIPS

Very few of the human studies of radiation carcinogenesis permit examination of a range of dose-effect relationships from high to low dose levels. We present two examples, one related to leukaemia in Japanese atomic bomb survivors and the other to thyroid cancer from X-ray therapy and other sources.

Figure 1 shows graphically the relation between total radiation dose and annual incidence rates of leukaemia for the 16-year period 1950-1966 among atomic bomb survivors in Hiroshima, as reported by Ishimaru et al. [3]. Incidence rates, unadjusted for sex and age, were computed for the following dose ranges (in rads): under 5, 5-19, 20-49, 50-99, 100-199, 200-299 and 300+. It is clear that leukaemia rates increase as a function of increasing dose.

In Fig.2, crude rates of malignant thyroid tumours in persons irradiated in infancy and childhood are plotted according to mean thyroid dose. The rates are derived from six epidemiologic studies with mean follow-up periods of 16-25 years and widely varying thyroid doses [11]. The studies include follow-up of those irradiated for thymic enlargement in the Rochester, New York, area and Ann Arbor, Michigan, investigated by Hempelmann and colleagues [12]; the study of X-ray epilation for tinea capitis in Israel by Modan et al. [7]; the study of A-bomb survivors in Hiroshima and Nagasaki, reported by Jablon and others [13]; and the follow-up of those exposed to radioactive fallout in the Marshall Islands by Conard and colleagues [14]. Mean thyroid doses of 6.5 up to 1225 rads are rather consistently associated with concomitant increases in frequencies of thyroid cancer cases, ranging from 1.1 to 52.6 per 1000 irradiated.

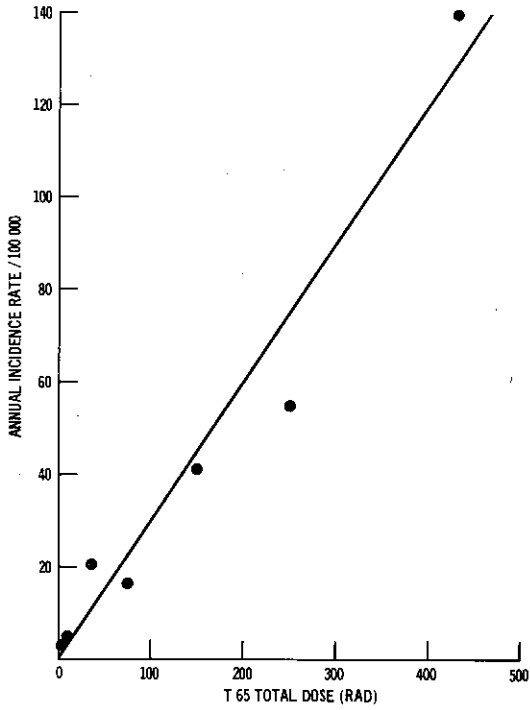


FIG. 1. Annual incidence rates of leukaemia per 100 000 A-bomb survivors by tentative 65 total dose, Hiroshima, Oct. 1950 to Sep. 1966 (rates from Ishimaru et al. [3]).

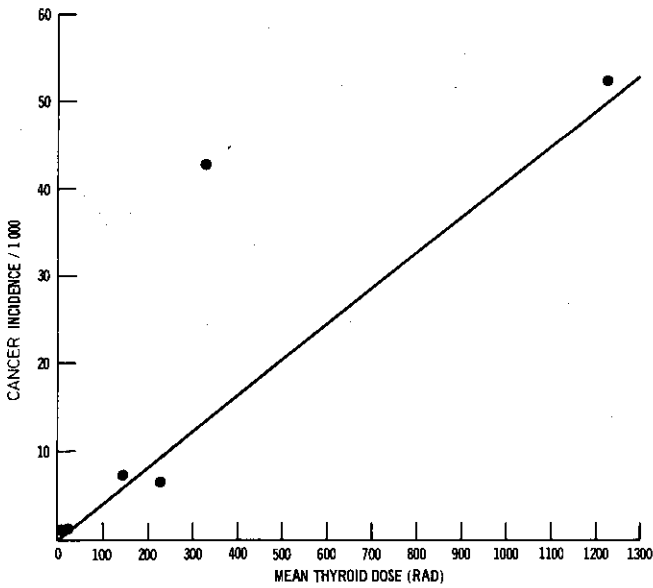


FIG. 2. Crude rates of malignant thyroid tumours per 1000 persons irradiated in infancy and childhood, by mean thyroid dose (rads) — selected studies (unpublished data from Silverman and Hoffman, 1975).

To our knowledge, there are no other reported situations in man that can demonstrate dose-effect correspondence down to very low levels at this time. The Atomic Bomb Casualty Commission (ABCC) study of leukaemia in the Hiroshima population represents the only study with appropriate dose groupings to report late carcinogenic effects over a wide dose range extending below 50 rads. The dose-effect relationship for thyroid cancer is derived from the findings of six studies of infants and children.

POSSIBLE ADDITIONAL SOURCES OF INFORMATION

Studies of irradiated human populations which have potential for yielding cancer risk information at low levels are of two types: (a) those which can provide further data about effects in the low-dose range; and (b) those which can demonstrate dose-effect relationships from high to low-dose levels.

(a) Low-level exposure studies

In this category are studies which can, with further analysis, investigation or follow-up, provide more precise risk figures for several types of cancer in several population groups.

The tinea capitis scalp X-irradiation studies in Israel and in New York City can do this for specified malignancies of the head and neck region in persons irradiated in childhood. In Israel, ongoing additional follow-up will extend the period of observation and provide further data on the increased incidence of thyroid cancer associated with an estimated mean thyroid dose of 6.5 rads. (The current follow-up effort will also furnish data on thyroid adenomas so that the relationship between the occurrence of malignant and benign thyroid tumours can be examined over a 25-year period in a large population.) In the same study, if planned additional dosimetry includes the parotid gland, it can be determined whether the 39-rad dose found in the New York City study is applicable to the excess of parotid cancer found in Israel. In New York City, further information about low-dose radiation-associated skin cancer may become available in time; the 29-year cumulative rates for all tumours indicate that incidence is rising continuously with post-treatment time.

The relationship between prenatal diagnostic irradiation and increased risk of childhood cancer is being investigated further by the National Academy of Sciences - National Research Council [15]. The study, which involves children born in military hospitals, will extend the findings of the Oxford Survey of Childhood Cancers [10] and avoid biases of socioeconomic influence on the use of X rays and mothers' recollection of X ray examination during pregnancy.

The Co-operative Thyrotoxicosis Follow-up Study of patients treated for hyperthyroidism during 1946-1964 with large doses of ^{131}I and other therapies reported, after a mean follow-up period of 6.5 years of the irradiated group, no evidence of radioiodine-induced leukaemia [16]. The mean whole-body dose was approximately 11 rads, the blood dose about 15 rads, and the bone-marrow dose range 7-13 rads. Reopening the study

to allow a longer period of follow-up would provide further information about possible leukaemogenesis and other carcinogenic effects of this internal emitter at low doses.

(b) High- and low-level exposure studies

The ABCC investigations in Japan span a broad range of doses and include a wide variety of malignancies. Many new cases of cancer have developed in recent years. To date, increases in malignancies other than leukaemia have been reported to be associated with large radiation doses (90+ rads). Significant increases of salivary gland malignancy [17] and thyroid cancer as well as probable increases in breast and lung cancer have been reported [2]. Continued observation and specification of lower dose categories may in time reveal dose-effect relationships in addition to those for leukaemia.

A large nationwide study has been undertaken by the Canadian National Cancer Institute to investigate former hospitalized tuberculosis patients exposed to radiation during repeated fluoroscopic examinations for pneumo-therapy many years ago [18]. It is believed that the risk of cancer induction following exposures down to 40 - 100 rads should be quantifiable for leukaemia and lung cancer and probably also for breast cancer. Another similar study of former tuberculosis-sanatorium patients is being conducted in Massachusetts at the Harvard School of Public Health with support from the Bureau of Radiological Health [19]. In this smaller study, females exposed around puberty or as young adults are being followed up to determine breast cancer risks related to radiation dose and age at exposure.

An ongoing study at the Research Triangle Institute in North Carolina concerns the follow-up of young adults who were given diagnostic doses of ^{131}I as children prior to 1961. The objective is to define a dose-response curve in the range of 30 - 1000 rads for the development of thyroid neoplasms [20].

A study of the long-term effects of occupational radiation exposure to X-ray technologists, by the National Academy of Sciences - National Research Council [15], is an extension of an earlier study of Army-trained World-War-II technologists which found more lung cancer deaths in the exposed group than in the control group. Ten additional years of observation will be added to the previous 18 years of follow-up. Although precise doses cannot be determined, exposure was considered relatively small compared with that of radiologists, but substantially greater than that of the general population: perhaps at least a weekly dose of 0.1 - 0.3 R or 5 - 15 R per year [21]. Our Radiation Registry of Physicians study, a long-term follow-up of radiologists and comparable medical specialists in pathology [22], may provide some information about the effects of low as well as high exposures in an occupational group, particularly in younger radiologists.

SUMMARY

On the basis of study findings to date, the following observations can be made: (1) doses under 50 rads have been associated with cancer induction in population groups of varying age composition irradiated under

different circumstances; and (2) there is some evidence of dose-response relationships over a wide range of doses extending down to less than 10 rads.

Intra-uterine exposures from diagnostic X radiation with 0.5 - 2.0 rads are associated with the development of leukaemia in childhood and possibly also of solid tumours. X-ray epilation for tinea capitis in children has led to low-dose radiation-induced thyroid cancer, and possibly also parotid gland and skin cancer, after two to three decades.

The results of continued study of the Hiroshima population over a 16-year period are consistent with a linear dose-response relationship for leukaemia induction down to 20 - 49 rads. Data from six studies of head and neck irradiation during childhood, based on mean follow-up periods of 16-29 years, similarly indicate that the risk of developing thyroid cancer appears proportional to the thyroid dose, down to 6.5 rads.

Radiation factors other than dose, such as dose rate and type of radiation, have not been considered in this brief report. An earlier review of thyroid cancer risk from radiation during childhood revealed relatively uniform risks in different situations [11]. The estimated risks of thyroid cancer: 2.1 - 6.1 cases per million irradiated children per rad per year, were associated with different types of radiation (X ray, neutron and γ , and β and γ) and a wide range of thyroid doses (6.5 - 1225 rads). Further data are needed to determine the relationship between radiation dose, type of radiation, and cancer incidence.

Although not many studies capable of detecting low-dose carcinogenic effects in man have yet reported findings, there are reasonable expectations for further data as various studies are carried out or completed, or possibly initiated. This should provide improved estimates of cancer risks based on observations in the lower dose ranges.

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DISCUSSION

M.L. GRIEM (Co-chairman): Mammograms are being recommended for breast cancer screening and detection. Since radiation is known to induce breast cancer, has a risk-benefit ratio been assigned to this procedure? What practical advice can you give us concerning this examination, and when does it induce more cancers than it detects?

Charlotte SILVERMAN: These questions are currently under active investigation, particularly since age at screening and the type of equipment used are involved. Women in the 35-50 age group are the subject of the current benefit/risk studies.

M.H. MOMENI: It is possible that at lower exposure levels biological response to radiation may be related to the heterogeneity of the population, i.e. different sub-groups of the American population may show responses which are genetically related. An example of the genetically related response to radiation is skin cancer, where it has been shown to have a higher probability of incidence in the fair-skinned sub-group. Is any study being carried out at present to identify the relationship between the population sub-groups and the type of cancer at lower levels of radiation exposure?

Charlotte SILVERMAN: This should become possible as more information is gained about the carcinogenic effects of low-dose radiation. Some factors which are known to influence the "natural" frequency of several types of cancer are accentuated in their effects by radiation.

R. WILSON: A source of information on risk that is becoming available is exposure of nuclear power station workers. The current occupational dose at US light-water reactors is about 1 man·rem/MW(e) installed. In the next few decades the dose to this group of workers will be in the range of 20 000 to 100 000 man·rem/year. Prospective studies should be undertaken now; dose records, medical records, etc., should be planned in order to extract and simplify data. This is being done at Ontario Hydro.

Invited paper

PRESENT AND FUTURE RESEARCH
PROGRAMME OF THE RADIATION EFFECTS
RESEARCH FOUNDATION (RERF)
Formerly the Atomic Bomb Casualty
Commission (ABCC)

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Abstract

PRESENT AND FUTURE RESEARCH PROGRAMME OF THE RADIATION EFFECTS RESEARCH FOUNDATION (RERF): FORMERLY THE ATOMIC BOMB CASUALTY COMMISSION (ABCC).

A summary of major scientific achievements so far obtained by the Atomic Bomb Casualty Commission (ABCC) on the long-term effects on man of atomic bomb exposure are presented as well as research programmes to be carried out by the newly established Radiation Effects Research Foundation (RERF), which has replaced the original ABCC.

Based on my experience of seven months as Chairman of the Board of Directors of the Radiation Effects Research Foundation (RERF), I should like to describe the present state and future prospects of the RERF programme.

The Atomic Bomb Casualty Commission (ABCC) was reorganized as the RERF under Japanese law in April 1975. The RERF is financed equally by the governments of Japan and the United States of America, with the participation of the Ministry of Health and Welfare (Japan), the Ministry of Foreign Affairs (Japan), the United States Energy Research and Development Administration and the National Academy of Sciences (USA). An equal number of board members and scientific councillors were appointed from each country. The present members of the Board of Directors are listed in Appendix A while those of the Scientific Council are shown in Appendix B.

On 15 April the first meeting of the Board of Directors was held and from 10 to 12 July the first meeting of the Scientific Councillors took place, followed by the second Board Meeting from 24 to 26 September. As a result of these meetings the policy of research and the basis for budgetary requests were decided. During the initial stage the RERF will continue the ABCC programmes using the samples outlined in Fig.1.

According to our Act of Endowment, the Scientific Council formulates the general outline of research programmes and the plan of activity, which will be submitted for review and approval by the Board. The Board of Directors will then plan the methods for implementing the recommendations made by the Scientific Council, including budgetary matters and operation policies. As we are dependent upon the two governments for our budget and as their approval and endorsement are required, it is not yet known to what

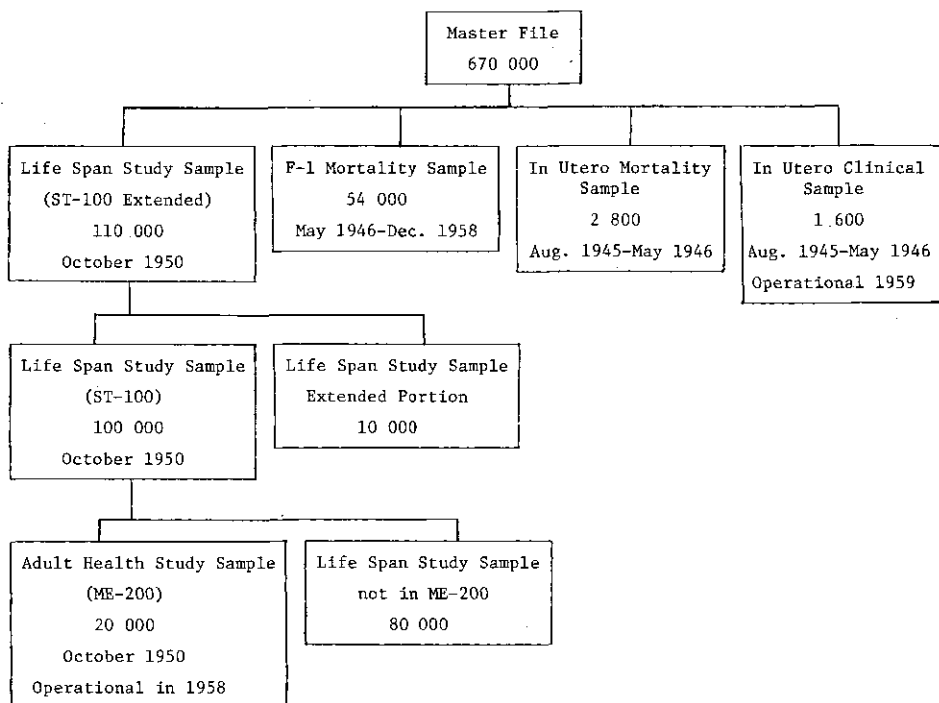


FIG. 1. Major ABCC sample sizes and date sampled (Hiroshima and Nagasaki combined).

extent our proposals will be approved for us to put into effect. Though it is not possible to make any conclusive statement about the distant future, I will present a brief outline of our present research programme and future prospects.

In February 1975 the Crow Committee organized by the National Academy of Sciences made a scientific review of the ABCC programme made up as follows:

- Life Span Study (LSS)
- Adult Health Study (AHS)
- Pathology - Autopsy Programme
- Cytogenetics
- Cancer Studies
- Genetics
- In-Utero Radiation Studies
- Aging Studies
- Dosimetry

The Scientific Council, which had its first meeting in July 1975, made the following recommendations regarding the research programme.

TABLE I. COMPARATIVE ESTIMATES OF ABSOLUTE AND RELATIVE RISK, MAJOR CANCERS (from Beebe and Kato, Ref. [4])

Type of cancer	Age ATB	Incidence (I) or Mortality (M)	Time Period	Cases per 100,000 per year			Relative risk*		Excess cases per 10 ⁶ PYR#	
				0-9 rads	10+ rads	100+ rads	10+ rads	100+ rads	10+ rads	100+ rads
Leukemia	All	M	'50-'72	4.4	17.6	51.2	4.0	11.6	1.6	1.9
Thyroid	All	I	'50-'71	18.9	35.6	38.5	1.9	2.0	1.2	0.8
Breast	10	I	'50-'69	24.4	41.0	72.8	1.7	3.0	2.1	2.0
Lung†	35	M	'50-'72	41.2	57.4	83.9	1.4	2.0	2.0	1.8

* For the dose group shown, relative to the risk of those exposed to 0-9 rads

Excess cases above expectation calculated from rates for those exposed to 0-9 rads, expressed per million person·year·rads (PYR).

See BEIR report pp. 195-199.

† Not corrected for incomplete reporting on death certificate.

1. CLINICAL PROGRAMME - ADULT HEALTH STUDY (ME-200)

The Adult Health Study (AHS) is a morbidity study of A-bomb survivors and their controls based on biennial physical examination. It was recommended that efforts should be made to enhance efficiency without sacrifice of scientific productivity, and that further studies should be conducted on morbidity and other delayed effects with greater emphasis on those exposed to over 100 rads.

Table I shows the comparative estimates of absolute and relative risk of leukaemia and major cancers by exposure dose. The incidence of leukaemia and these four cancers showed a significant increase proportional to the estimated doses received.

(a) Cancer of the thyroid

The occurrence of thyroid cancer was higher in women than in men and showed a significant increase proportional to the estimated radiation dose received. Incidence was increased in those exposed to over 200 rads in the oldest age group ($p < 1$). Thyroid cancer was significantly more prevalent in women exposed to 50 rads or more ($p < 0.001$). Cancer of the thyroid diagnosed during life was more common in persons who were less than 20 years old at the time of A-bomb exposure (ATB).

(b) Cancer of the lung

Cancer of the lung was increased above normal expectation in persons who received 90+ rads in Hiroshima and Nagasaki. The ratio of observed to expected cases was 1.6 and the findings suggested an increased risk of lung cancer following irradiation.

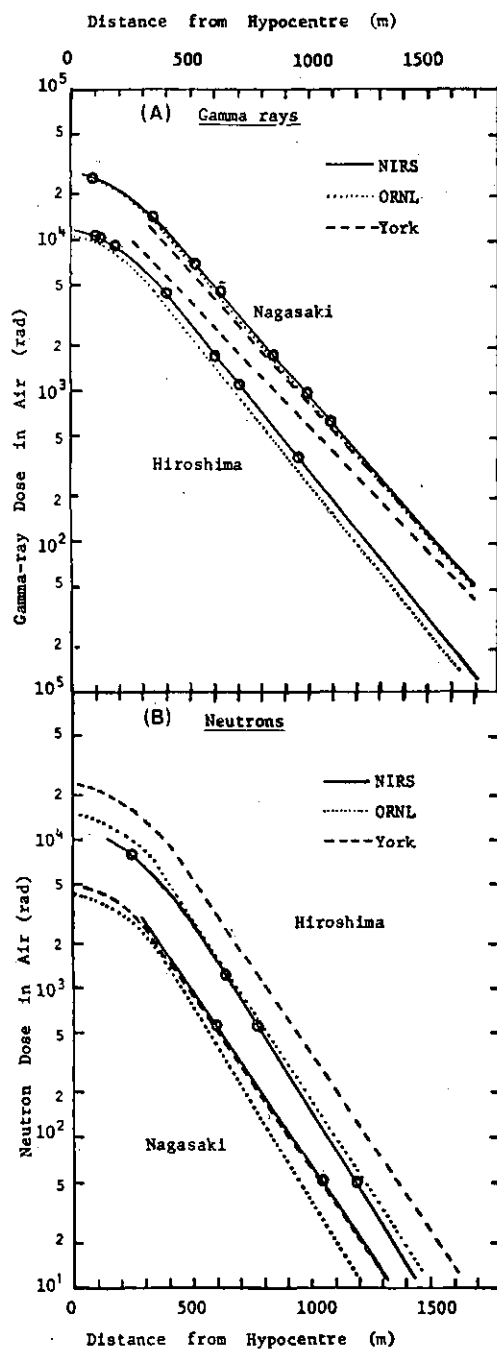


FIG. 2. In-air tissue-absorbed dose due to γ rays (A) and neutrons (B) in Hiroshima and Nagasaki as a function of distance from hypocentres. Full line with open circles shows γ -ray dose (A) and neutron dose (B) estimated by NIRS. Dotted line shows γ -ray dose (A) and neutron dose (B) by York. Broken line shows γ -ray dose (A) and neutron dose (B) by ORNL. (From Hashizume and Maruyama, Ref. [7]).

(c) Cancer of the breast

Women exposed to 90+ rads developed breast cancer at two to four times the rates observed in the comparison groups of the sample and the reported rates for Miyagi Prefecture.

A substantial portion of AHS findings may be called negative. Findings have been negative for aging, fertility, cardiovascular diseases, cerebrovascular diseases, and clinical diseases associated with chromosomal abnormalities.

2. PATHOLOGY PROGRAMME

The need to establish closer ties with the local universities, major hospitals and other medical treatment organizations and to make efforts to collect as much data as possible was pointed out. Efforts should be made to perform autopsies primarily on those who die at home, and assistance should be extended to the Leukaemia Registry, Tumour Registry and Tissue Registry programmes.

3. DOSIMETRY

It was noted that through the research work of Dr. Auxier (Oak Ridge National Laboratory), Dr. Hashizume (National Institute of Radiological Sciences) and others, individual dose estimates have become available.

Figure 2 shows the in-air tissue-absorbed dose due to γ rays (A) and due to neutrons (B) in Hiroshima and Nagasaki. Full lines with open circles show dose estimated by Dr. Hashizume (NIRS), the dotted lines by Drs Ritchie and Hurst (York) and the broken lines by Dr. Auxier (ORNL). In the case of these dose estimates, ABCC data have been recalculated according to estimated exposure doses and not according to distance from the hypocentre as in the past. A few examples will be given.

Figure 3 shows percentage of Hiroshima children exposed before 18th week of gestation with small head circumference according to radiation dose (reported by Dr. Blot). Figure 4 shows crude annual incidence rate of leukaemia in Hiroshima (shaded) and Nagasaki by exposure dose between October 1950 and December 1971. It is evident that the risk was greater in Hiroshima than in Nagasaki in every dose category over 50 rads and that the risk increased with dose. Figure 5 shows that the dose-response relationship differed by type of leukaemia. The risk of acute leukaemia is elevated for those who received 100 rads or more. Figure 6 shows a schematic model of influence of age at the time of bombing and calendar time on leukaemogenic effect of radiation in heavily exposed survivors. The risk of chronic granulocytic leukaemia was the greatest in the youngest age group.

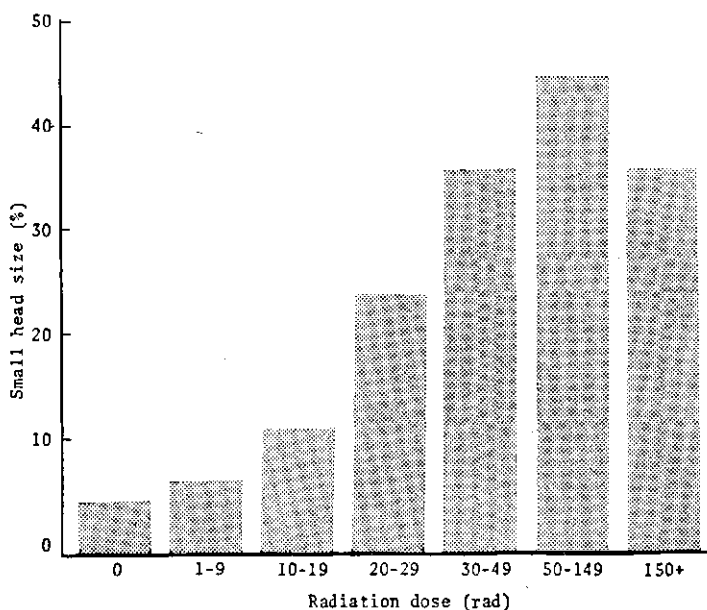


FIG. 3. Percentage of Hiroshima children exposed before 18th week of gestation with small head circumference according to radiation dose (from Blot, Ref. [6]).

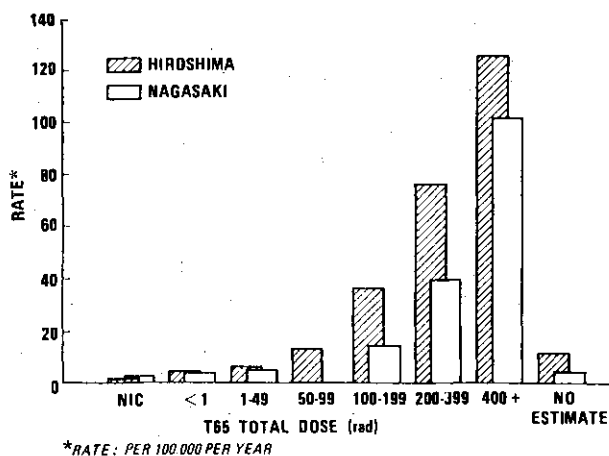


FIG. 4. Crude annual incidence rate of leukaemia (all forms) in the extended Life Span Study sample by city and dose, Oct. 1950-Dec. 1971 (from Ichimaru and Ishimaru, Ref. [8]).

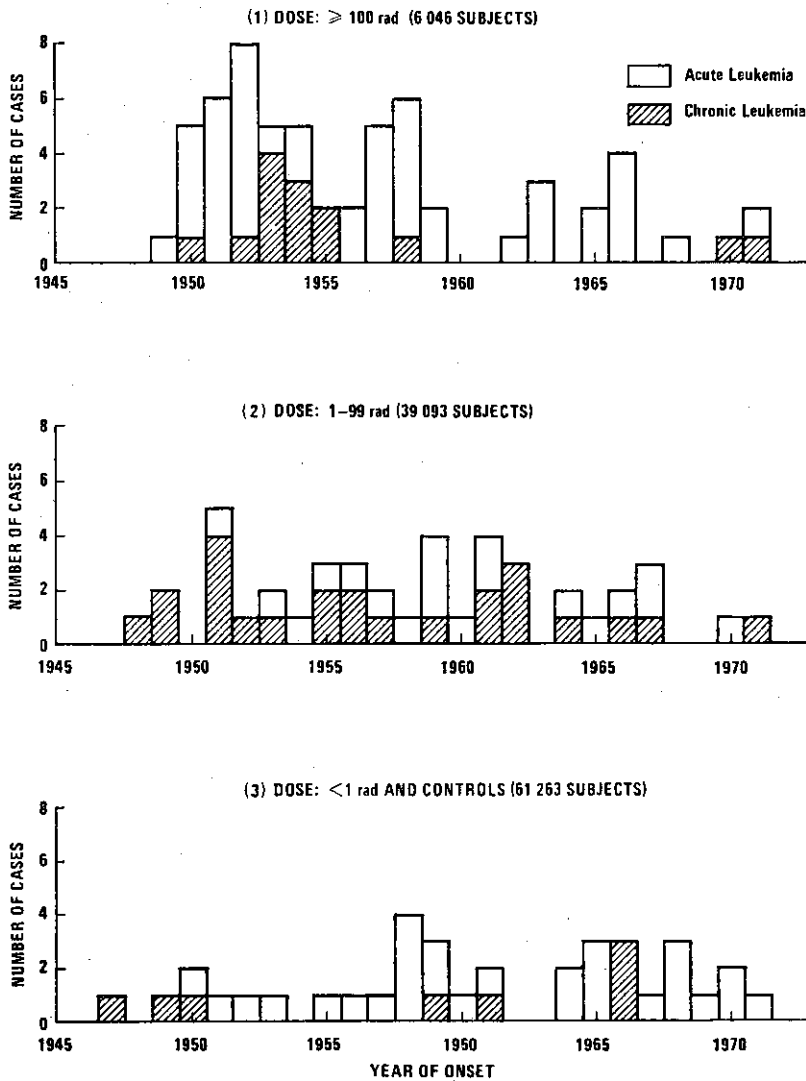


FIG. 5. Distribution of definite and probable leukaemia in the fixed cohort of atomic bomb survivors and controls, Hiroshima and Nagasaki, by year of onset, dose and chronicity of leukaemia (from Ref. [8]). The cohort was established on 1 Oct. 1950.

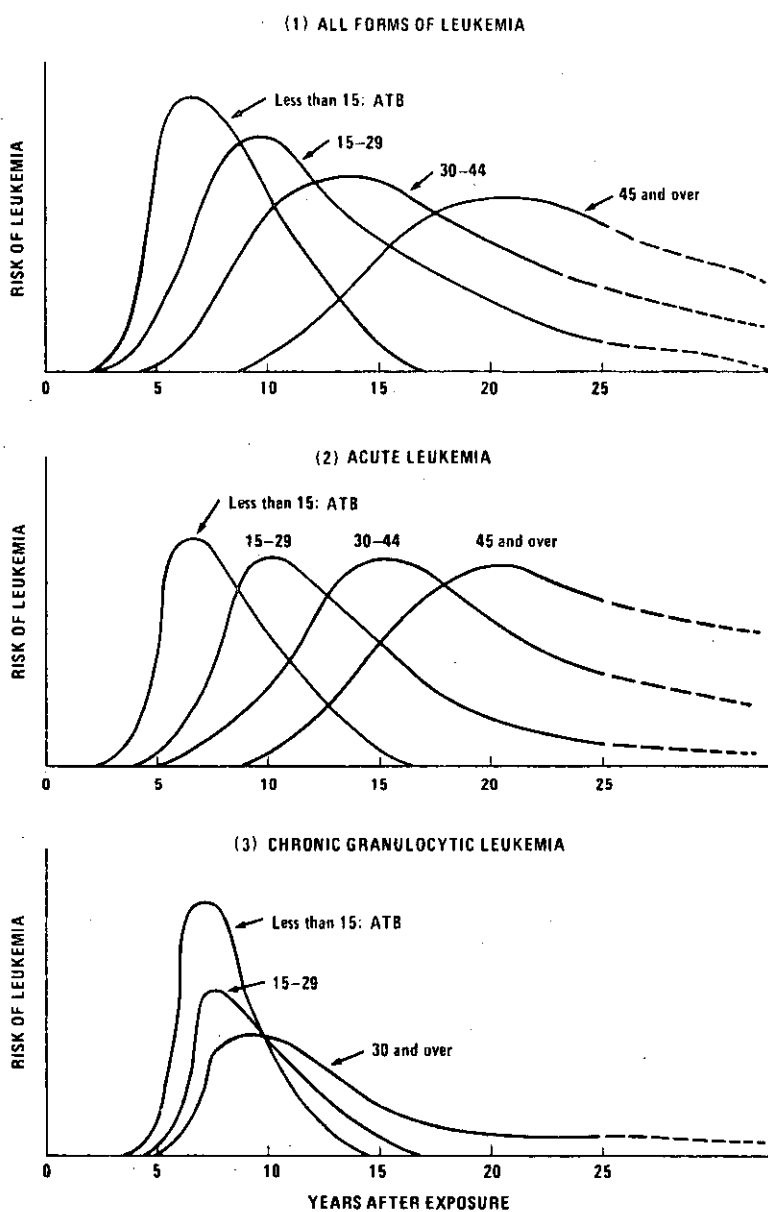


FIG. 6. Schematic model of influence of age at time of bombing (ATB) and calendar time on-leukaemogenic effect of radiation (heavily exposed survivors). (From Ref. [8].)

Table II. Cytogenetic findings in Hiroshima and Nagasaki A-bomb Survivors*

Exposure group (rad)	Mean dose (rad)	Sub-jects	Cells Ob-served	Number of cells with				Total	Total exchanges
				dic + r	ace	t + inv	del		
Hiroshima									
Control	—	134	13289	24(0.18)	28(0.21)	80(0.60)	12(0.09)	144(1.08)	106(0.80)
Total exposed	252.4	130	12852	74(0.58)	34(0.26)	809(6.29)	71(0.55)	988(7.70)	893(6.95)
100-199	144.9	59	5859	24(0.41)	15(0.26)	199(3.40)	20(0.34)	258(4.40)	226(3.86)
200-299	243.0	36	3574	20(0.56)	7(0.20)	226(6.32)	23(0.64)	276(7.72)	248(6.94)
300-399	352.8	18	1750	18(1.03)	4(0.23)	157(8.97)	12(0.69)	191(10.91)	178(10.17)
400-499	442.2	11	1069	8(0.75)	4(0.37)	117(10.94)	10(0.94)	139(13.00)	126(11.79)
500+	716.5	6	600	4(0.67)	4(0.67)	110(18.33)	6(1.00)	124(20.67)	115(19.17)
Nagasaki									
Control	—	79	7418	17(0.23)	17(0.23)	25(0.34)	10(0.13)	69(0.93)	44(0.59)
Total exposed	244.8	113	10478	28(0.27)	32(0.31)	203(1.94)	29(0.28)	292(2.79)	236(2.25)
100-199	142.3	47	4423	11(0.25)	11(0.25)	33(0.75)	6(0.14)	61(1.38)	44(0.99)
200-299	246.7	40	3791	9(0.24)	14(0.37)	66(1.74)	14(0.37)	103(2.72)	79(2.08)
300-399	350.4	12	1051	3(0.29)	3(0.29)	12(1.14)	1(0.10)	19(1.81)	15(1.43)
400-499	436.1	10	847	4(0.47)	3(0.35)	46(5.43)	8(0.94)	61(7.20)	51(6.02)
500+	636.0	4	366	1(0.27)	1(0.27)	46(12.57)	0	48(13.11)	47(12.84)

dic: dicentric (and multicentric); r: ring; ace: acentric fragment; t: reciprocal translocation; inv: pericentric inversion; del: deletion

* Cited from Awa et al. (Ref.[3])

Numbers in parentheses indicate percentage of aberrant cells.

4. CYTOGENETICS PROGRAMME

Two major cytogenetics projects are currently being carried out at the Radiation Effects Research Foundation; one on the effects of A-bomb irradiation on the somatic cell chromosomes of survivors and the other on the effect of A-bomb irradiation on the germ cell chromosomes of the exposed that may influence genetic risks to offspring.

Persistent chromosomal aberrations are observed in the cultured peripheral lymphocytes from A-bomb survivors nearly three decades after radiation exposure (Table II). As shown in Fig.7, the frequency of such aberrant cells is roughly proportional to the exposure dose. In general, the frequency of cells with induced aberration is consistently higher in Hiroshima than in Nagasaki for every dose category, suggesting stronger effects of neutron than γ radiation for induction of chromosome aberrations. Biological and clinical implications of cells with radiation-induced chromosome aberrations remain to be further explored.

The somatic chromosomes of the children of A-bomb survivors have been investigated to see whether there is any evidence of radiation damage in the germ cell chromosomes of the exposed. Based on this assumption, we have examined approximately 3000 children born to either one or both parents whose estimated doses were more than 1 rad, and about 1000 children of the non-exposed parents as controls. Although we have observed a

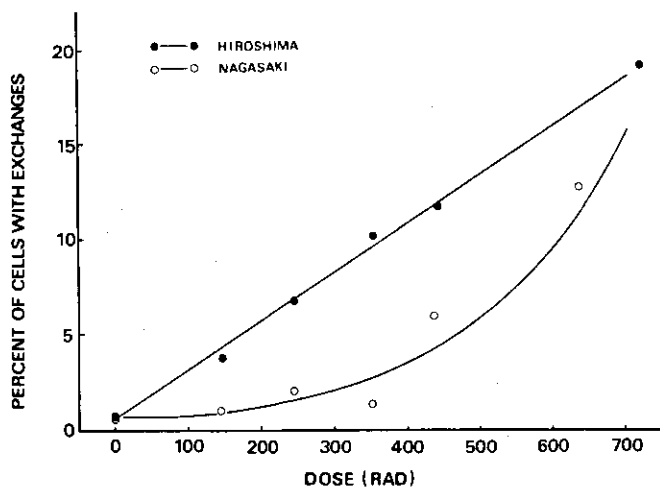


FIG. 7. Chromosome aberrations (from Awa, Ref. [3]).

slightly increased frequency of individuals with sex chromosome abnormalities as well as with balanced reciprocal translocations in the exposed group, no statistical difference has to date been demonstrated between the controls and the exposed (Table III). More cases must be studied before any conclusion can be drawn.

5. BIOCHEMICAL GENETIC STUDY

Recent advances in electrophoretic techniques for the detection of isozymes have made it possible to detect various genetically determined biochemical variants in the human population. By using these techniques it may be possible to evaluate the genetic effects of A-bomb radiation-induced mutations by obtaining the rate of changes in these biochemical parameters. Table IV shows the number of variants detected among the 5529 sample members of the ME-200 and F_1 populations in Hiroshima and Nagasaki. By means of electrophoretic techniques, variants including T_f and PGM_1^7 were found at the rate of 3 out of 1000 cases. Phosphoglucose mutase is the system which showed the highest incidence of variants. Furthermore, a family study is in progress to determine whether these are genetic variants or not.

Since this is an important approach, we propose to conduct the study as a major research programme from 1976. A research plan to study 20 000 F_1 offspring will be established to determine whether there is any significant difference.

6. F_1 STUDIES

Pathological and lifespan studies undertaken to date on the F_1 population have indicated no increased risk in the mortality rate among the offspring

Table III. Comparison of cytogenetic data on (A) induced abortuses, (B) newborn infants, and (C) children of A-bomb survivors (from Awa, Ref. [2]).

	(A) Induced abortuses	(B) Newborn infants	(C) F ₁ of survivors	
			Control	Exposed
Total examined	1129	46058	1090	2885
Male	579	29885	510	1386
Female	550	16173	580	1499
<u>Sex anomaly</u>				
XXY	1(0.17)	31(0.10)	1(0.20)	3(0.22)
XYY	—	26(0.09)	—	3(0.22)
Other	—	21(0.07)	—	—
Subtotal	1(0.17)	78(0.26)	1(0.20)	6(0.43)
X	5(0.91)	2(0.01)	—	—
XXX	—	13(0.08)	—	2(0.13)
Other	—	8(0.05)	—	1(0.07)
Subtotal	5(0.91)	23(0.14)	—	3(0.20)
Total	6(0.53)	101(0.22)	1(0.09)	9(0.31)
<u>Autosomal trisomy</u>				
+C	1(0.09)	—	—	—
+D	2(0.18)	3(0.01)	—	—
+E	2(0.18)	4(0.01)	—	—
+G	3(0.27)	49(0.11)	—	—
Other	2(0.18)	2(0.00)	—	—
Total	10(0.89)	58(0.13)	—	—
<u>Structural anomaly</u>				
t(D/D)	—	31(0.07)	—	3*(0.10)
t(D/G)	—	9(0.02)	—	1(0.04)
Balanced	1(0.09)	45(0.10)	2(0.18)	5(0.17)
Unbalanced	—	19(0.04)	—	—
Other	—	5(0.01)	—	—
Total	1(0.09)	109(0.23)	2(0.18)	9(0.31)
<u>Polyploidy</u>				
Triploid	1(0.09)	—	—	—
Grand total	18(1.59)	268(0.58)	3(0.28)	18(0.62)

(A) from refs. [10] and [11]. (B) from refs. [12–22], for details, see ref. [20].

* Two are sibs.

TABLE IV. VARIANTS FOUND FROM JULY 1972 TO 31 AUGUST 1975

System	ME-200	F ₁	Total
Alb	10	2	12
Crpl	2	3	5
Hp	1	2	3
Tr	84	37	121
Hb A	2	0	2
Hb A ₂	2	0	2
ADA	0	1	1
CAI	4	3	7
ICD	1	3	4
LDH	1	0	1
MDH	1	0	1
Pep A	6	4	10
Pep B	8	1	9
6PGD	4	0	4
PGM ₁ PGM ₂ Others	54 20) 74	30 10) 40	84 30) 114
PHI	35	11	46
Total	235	107	342
Total Number	4029 (H 2653 N 1376	1500	5530

of A-bomb survivors in comparison with those of the non-exposed controls. Recently, this genetic programme has been expanded to include the above cytogenetic and biochemical genetic studies. Since family studies will be necessary for the conduct of the latter, efforts would have to be made to obtain the consent of A-bomb survivors.

7. IMMUNOLOGY PROGRAMME

Immunological studies in relation to exposure dose are also of interest and should be promoted by all means, but many problems are involved. It is planned to hold a workshop to discuss plans for this programme on the occasion of the International Congress of Haematology to be held in Kyoto in September 1976, when many scientists concerned will assemble.

8. RENOVATION OF RESEARCH FACILITIES AND RECRUITMENT OF RESEARCH STAFF

The ABCC has a history of 28 years and its research facilities are on the whole superannuated, but up-dating of equipment has not been made to

any great extent. It is essential to modernize the research facilities, including the computer. To introduce new research projects, it is necessary to recruit outstanding research personnel from the United States of America and Japan.

We have an equipment budget of only 50 million yen for the fiscal year 1975, but 150 million yen have been requested for the fiscal year 1976. Because of the unusual economic difficulties facing Japan, our request for 1976 is very conservative. For the fiscal year 1977 we would want to increase the amount further.

9. CONCLUSION

The RERF is charged with the mission of investigating the late effects in the survivors of the atomic bomb and also the presence or absence of genetic effects in their F_1 offspring.

The projects undertaken at RERF are follow-up studies of very long duration on persons who have survived a single exposure to the atomic bomb at doses which were not necessarily of low level. These are very important studies whatever the findings may be.

We look forward to the continued support of scientific circles in the United States of America and Japan and of the entire world in the conduct of our research programme.

APPENDIX A

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 State University of New York at Stony Brook (Experimental Pathology)

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Invited paper

THE TRANSITION FROM THE ATOMIC BOMB CASUALTY COMMISSION TO THE RADIATION EFFECTS RESEARCH FOUNDATION

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Abstract.

THE TRANSITION FROM THE ATOMIC BOMB CASUALTY COMMISSION TO THE RADIATION EFFECTS RESEARCH FOUNDATION.

The events resulting in the establishment of the new foundation (RERF) to replace the Atomic Bomb Casualty Commission (ABCC) are briefly described.

Dr. Yamashita, in the foregoing paper, has provided a succinct but complete description of the Radiation Effects Research Foundation (RERF) and its programmes, and it would be redundant to attempt to elaborate. Thus an effort will be made to describe the chain of events that resulted in the establishment of the new foundation to replace the old Atomic Bomb Casualty Commission (ABCC).

It has been apparent since the inception of the ABCC and earlier that the Japanese experience in Hiroshima and Nagasaki represents a unique source of quantitative information on the effects of radiation on man. It is everyone's strong hope that this experience shall remain unique. Therefore, every effort has been and should be made to study these populations thoroughly for many years. This important function was carried out effectively for many years by the ABCC, supported by the United States of America and operated under US direction with the generous aid and advice of Japanese colleagues.

In recent years two trends developed that gave rise to a re-examination of the administrative arrangements required to pursue these studies. It was felt by both the Japanese and Americans that there should be more participation by the Japanese, not only in determining the breadth and scope of the scientific programme, but in terms of actual programme participation by Japanese scientists and physicians as well. Also, financial problems made it increasingly difficult for the Division of Biomedical and Environmental Research of the United States Atomic Energy Commission (now the Energy Research and Development Administration) to fund the studies at a level adequate to conduct the necessary scientific investigations. The financial problems were aggravated by inflation, the changing relative value of Japanese and American currencies, and the fact that the expanded mission of USERDA introduced many new demands on a budget that was not increased to a degree commensurate with the broadened responsibilities.

As a result of these considerations, a series of discussions was initiated between Japanese and American officials, and an Act of Endowment for the new Radiation Effects Research Foundation was worked out. The discussions culminated with the signing of the Act of Endowment in Tokyo in February 1975.

The Radiation Research Effects Foundation became a reality on 1 April 1975, at which time the old ABCC ceased to exist. All ABCC properties were turned over to RERF, and the new organization began to function as of that date. Key to the spirit and operation of the new Foundation is the equal participation of Japanese and Americans in the establishment of the policy for this organization, its operation and its financial support. The Board of Directors has equal representation from Japan and the United States of America, as does the Board of Scientific Councillors. The Board of Directors met once and the Board of Scientific Councillors twice in the calendar year 1975. Thus the organization is now well established and operating.

The RERF represents a sizeable operation with extensive responsibilities in two cities. An excellent nucleus of scientific and other staff has carried over from the ABCC. Significant additions have been made to the scientific staff, and the outlook for carrying on the programme appears bright.

It was clear from the start that the new foundation had complete support at all levels of operation. The Scientific Councillors have worked well together and have made very constructive suggestions with respect to the scientific programme. Similarly, the Board of Directors has worked in unison to take care of the many problems posed by RERF, as with any new developing organization. Importantly, the Governments of both nations involved have given every indication of complete support for the new venture. It is significant that the local scientific, medical and other organizations in Hiroshima and Nagasaki have indicated willingness to do whatever they can to ensure that the new organization will be a continuing success.

It is evident already that significant changes in the scientific programme will be brought about. There has been substantial interest in studies on the genetic effects of radiation, particularly because of the almost total absence of this type of data on the human being and because of the essentially unique resource in this respect to be found in the exposed populations in Hiroshima and Nagasaki. In recent years very sensitive biochemical approaches to the determination of genetic changes have been developed and automated. It seems clear that this type of programme, holding high promise of yielding definitive quantitative information on genetic effects of radiation in man, may be put into operation in the very near future.

The establishment of RERF represents the breaking of new ground, in that the organization is operated and financed on an equal basis by two governments. Obviously, there are real and potential problems that must be and are being worked out — problems with personnel, finances and other matters. As is to be expected, the detailed mechanisms of conducting business differ in the two countries. However, it is clear in meetings of the Board, which are attended by Japanese officials, that there is real desire to see that these problems are resolved and that the Foundation will succeed. With this spirit of accommodation the success of the new venture appears to be assured.

GENERAL COMMENTS

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E.E. POCHIN: This symposium was to address itself to the biological effects of low-level radiation and to the protection of man and his environment. Quite rightly it has concentrated on the important question of the protection of man, and I think it is most impressive to note how many papers have made reference to a central and crucial problem of radiation protection, namely development of valid numerical estimates of the overall safety or risk of doses of a few rads or less, delivered within a year, in addition to natural background.

It is revealing that the direct epidemiological evidence on harm to man himself at these low doses has been summarized quite fully in two or three of the 72 papers presented at the symposium. The human race provides greater numbers than any mouse colony, and is very much better studied as regards health and mortality, but obviously the direct data on harm is very limited, being confined to effects on the thyroid in children, on various tissues in the foetus and on the bone marrow. We have data on induction of malignancies at doses below 10 rads, which are valid for the thyroid if the Israeli children kept their heads still during the irradiation of their scalp, and didn't too often get their thyroids in the direct beam (MODAN, B., et al., Radiation-induced head and neck tumours, *Lancet* 1 (1974) 277). If so, we have valid data down at 6.5 rads, and it is perhaps disturbing that the rate of thyroid cancer induction per rad is about the same as for the data of Hempelmann at about 350 rads. We have further data at a rather unknown dose, but certainly a low one, for tissues of the foetus, again provided there were no biasing factors, and the observations on twins tend to exclude such bias (MOLE, R.H., Antenatal irradiation and childhood cancer: causation or coincidence? *Br. J. Cancer* 30 (1974) 199). And we have data on bone marrow at low doses of 0.9 rads provided again that the more extensive survey of larger exposed populations at Hiroshima and Nagasaki supports the numerical data obtainable on the groups already published (MORIYAMA, I.M.,

KATO, H., Mortality Experience of A-Bomb Survivors 1950-72, 1970-72; JNII-ABCC Life Span Study Report 7, Technical Report 15-73). Quite clearly, though, we are obtaining no general estimate of cell sensitivity to cancer induction by low doses of radiation, if only because the thyroid is about the most susceptible organ for cancer induction and its mass is only 20 grams, of which the greater part is cell-free colloid.

It is, however, very striking how information has been converging upon this central problem in the remaining papers, both from data at low dose on other species and from information on the mechanisms of carcinogenesis by radiation at low doses which may allow better inferences from moderate doses to low doses. The comparison of effects in different species is extremely important in order to bridge the huge gap between the very similar chemical structure of biological material in man and other animal species and the enormously different frequency of expression of cancer in different species and even in different strains of animals following irradiation.

But it is valuable also that there has been such detailed study and discussion of mechanisms, and the increasing evidence for the presence of a quadratic term in dose-effect relationships for low LET irradiation is very important in terms of radiation protection philosophy and procedure. Here we have two quite separate problems: one is to estimate the most likely risk of harm at low doses, on biological criteria, and the second is to make a quite different estimate, namely, that which can be asserted to give, for protection purposes, the maximum likely value for the harm which might result from low doses. There is a constant need to narrow the gap between what we need to say for safety's sake in protection and what we believe biologically to be the most valid estimate.

It is very important, therefore, that the evidence is strengthening for the presence of a quadratic term in the dose-effect relationship and hence for the increasing likelihood that, for protection purposes, the purely linear extrapolation from the frequency of effects at high doses will maximize the estimate of risk at very low doses. During the discussions several points have emerged, however, which call for caution here: the ^{224}Ra data on the greater effectiveness of protracted than of less protracted radiation; the greater effectiveness in some instances of low dose rates than of higher dose rates; and particularly the repeated evidence of longer latencies for tumours induced by low doses than by high, and therefore of the likelihood that information obtained at limited periods of time after exposure to high doses may underestimate the frequency per rad of tumours which might ultimately occur from lower doses.

I think there is great value also in the quantitative study of the linear and quadratic terms - and of the likely values of alpha, relative to beta, simply because most data in man from which quantitative epidemiological evidence is derived for carcinogenesis are obtained at levels of dose that are likely to be higher than the cross-over point where the quadratic term becomes more important than the linear one. This implies that, for data obtained from a point higher than this cross-over but lower than any maximum of the curve when cell-killing predominates, the inference from the linear extrapolation will be an overestimate.

But, there is another important aspect of linearity: the linear relationship which may obtain within the range of the various environmental exposures with which we are concerned. The evidence we have heard as to the probable values of these two coefficients does suggest that, in the case of doses of

0.1 rad per year from background — and of a few rads or millirads added from various artificial sources — the linear term for low LET radiation will probably dominate over the quadratic. Consequently, the frequencies of the effects from such sources can be thought of as being proportional simply to the dose from each. If so, and if the risks of effects are purely additive within this range, we can review the detriment from each source independently, without needing to consider interactions due to combined effects of doses from different sources. And here we finally come to the question which appeared on the programme, but which quite understandably was not dealt with except in a few papers — the comparison between harm from ionizing radiation and that from other physical and chemical pollutants. This is an extremely important point because, as has been stressed, if the biological importance of low doses of radiation can be evaluated and compared with that of other environmental factors, then it is possible to look objectively at whether the detriment, for example, from using nuclear sources of power production is greater or less than the likely detriment from using alternative sources of power; or, for that matter, the detriment of not using any source at all, and going without the added power. I believe that this symposium has been of direct value in strengthening the bases on which realistic evaluations can be made of the level of safety or harm associated with exposure to low doses of radiation.

G.M. WATSON: Radiological protection is concerned almost exclusively with the effects of small doses of radiation, and the papers presented during the week have brought out many points of relevance to this field. Our standards of radiological protection are rather arbitrary, and therefore evidence which may help to put these standards on a rational basis or to assess the real significance of small radiation doses is particularly welcome. In these respects, the symposium has been most valuable.

The sources of radiation to which man is exposed include diagnostic and therapeutic medical irradiation, and several papers dealt with this subject, in particular with the carcinogenicity of foetal exposure to X rays. Evidence hitherto presented on this point has not been fully convincing, since bias from selection is difficult to exclude, and differences of opinion were still apparent in the papers that were presented. However, as was pointed out in discussion, recent observations on the incidence of cancer in twins X rayed in utero suggest that foetal exposure is in fact carcinogenic. If this interpretation is accepted, we do now have some direct evidence of the carcinogenicity of small doses of radiation in man, and some support for extrapolation to small doses from effects observable only at high doses. Moreover, since there is no real reason to think that the foetus is especially sensitive to carcinogenesis, we should not exclude medical exposure in general from consideration when assessing the effects of low-level radiation.

At present there are major uncertainties in extrapolating to low doses from the quantitative observations which are possible only at high doses and dose rates. These uncertainties are often forgotten when the magnitude of effects on populations is estimated, but everyone accepts that unnecessary radiation exposure is undesirable, whatever function may be supposed to relate dose and effect. Since some exposure to radiation is inevitable, definition of standards of safety then requires a belief that there is some level of exposure which is "acceptable", and that it is a practical proposition to ensure that this level is not exceeded. Bearing in mind differences in the radiation sensitivity of different organs and the complicating effects of variation in radiation quality, the only practicable means of meeting this requirement has been to adopt the hypothesis of a linear relation between dose and effect, which has come in for a good deal of criticism at this meeting.

Assumptions of linearity and an acceptable limit imply the further assumption that somehow we know just what degree of risk is acceptable to people. Critics of nuclear development frequently take the line that no imposed risk is acceptable and that our estimates of risk are likely to be wrong in any case. The definition of what is an acceptable risk is probably not within our province, but we can scarcely dispute the second point. Perhaps fortunately for our peace of mind, I think most of us have not taken the linear assumptions too literally. We have, in fact, believed that there is a substantial safety factor in any calculations of low dose effects that are based on a linear dose-response relationship, and we believe that some much-publicized estimates of the effects of small population doses were a good deal too high. I think any of us with lingering doubts on this point will probably have had them dispelled by the paper on background radiation (SM-202/805) presented in this session. This and many other papers have suggested that the usual calculations are conservative.

The foregoing refers to external radiation of low LET which makes the largest contribution to any present risk. Unfortunately, there are areas where we are less confident. I refer here to the effects of internal irradiation, in particular from α emitters and, to some extent, also from β emitters — although the latter may not be a practical problem. It is evident from a number of the papers presented that we are rather uncertain of the effect of varying dose and dose rate on the incidence of cancer in persons with internal exposure. There clearly exist dose-rate effects for internal exposure, and there is some sort of relationship between dose and effect, but we are very uncertain of what these relationships are. No easy extrapolation from animals to man is possible for internal exposure, but several of the papers presented offer some prospect of improving this situation.

For radiation of high LET it seems probable that the assumption of linearity provides an adequate approximation to the true dose-response relationship, although this may not be true for all kinds of tumours and the observed responses may be modified by the effects of cell killing. The data available do not allow very precise definition of this relationship in any specific incidence but, for calculation of the risks associated with an intake of plutonium, the risk coefficients proposed by C.W. Mays provide a useful starting point.

The suggestion has been made that the yield of radiation effects may be related to dose by linear and quadratic terms; which of these terms is predominant depends on the quality of the radiation. If accepted, this

suggestion may have some implications for radiological protection which we should consider. Ordinarily, a radiation worker is allowed a maximum dose of five rem a year, and we can calculate from the usually accepted values for risk coefficients for carcinogenesis just what this means in terms of actual risk at the end of a working life of twenty or thirty years. We do not pay much attention to this figure for two reasons. One is that no ordinary radiation worker is likely to be allowed to receive anything like the maximum dose over twenty years. The second is that most of his exposure will have come from radiation of low LET, and therefore we think the calculated risk will be an overestimate. Now there is another worker whose case we may consider, the underground uranium miner whose exposure to radon and its daughters is limited in most countries to four working level months a year. We have risk data which allow calculation of the miner's prospective risk of lung cancer after a working life in the mines. This risk is of the same order as that calculated for other radiation workers, but there are two points we should note in the miner's case. First, he is likely to receive a large part, if not all, of the allowed exposure, for the simple reason that it is expensive to lower working levels in mines and the operator is not likely to lower them more than is required. Second, since the risk is associated with α irradiation, we think our calculation will not be an overestimate. In other words, the risk calculated for the miner may be a real one, whereas that calculated for an ordinary radiation worker may not, and I believe we should consider whether or not the two cases should be treated differently. In this connection, it was of considerable interest to hear a paper describing the experimental induction of lung cancer by radon daughter exposure. We hope this sort of work will be extended, particularly with reference to the effects of smoking, since the nature of the relation between smoking and lung cancer in uranium miners remains obscure.

Finally, let me say that the subjects discussed at this symposium are important for radiation biologists and for health physicists. If we do not come to terms with the problems that have been put forward and find acceptable solutions for them, our standards of radiological protection will continue to be arbitrary and subject to the kind of public criticism that they have attracted recently. Moreover, we want to be certain that we do not find we have a fresh set of human data to examine.

W.K. SINCLAIR: The symposium has dealt with many issues relating to the effects of low doses of radiation, though somewhat unevenly. It opened with an interesting session on genetic effects, but this important subject subsequently received relatively little attention. It then continued with two sessions on biological effects and one on theories and models devoted mainly to new somatic information on the effects of external radiation in a variety of species, from insects to dogs. Many authors concentrated on

dose-effect relations and the application of the theory of dual radiation action to these data. The volume of new information on internal emitters (β emitters, ^3H , etc.) and on α emitters (hot particle problems) is impressive, and Dr. Watson has already dealt with this subject.

Of the two final sessions, the first related to human exposures resulting from medical irradiation, at levels close to the natural background, and the second to risks resulting from exposure to low levels and to man-made sources.

In addition, we have had three special lectures; two concerned with the achievements of the ABCC project in Japan and its successor, the Radiation Effects Research Foundation (a joint Japanese-American venture); and the third concerned areas of high natural background in the world, embodying a rapporteur's account of a recent conference in Brazil. This latter is not included in these Proceedings since it is understood that a report of the conference will be published by the Brazilian Academy of Sciences.

In the light of this material, the principal issues relating to low-dose exposures to man and his environment seem to me to be:

- (1) Dose-effect relationships, and thus the means of extrapolating to low doses. This should include an appreciation of dose-rate dependence.
- (2) Extrapolation from other biological species to man, and hence comparison with epidemiological data in man.
- (3) The problem of relating scientific information on radiation exposure to acceptable risk for population and occupational exposures, and comparison of these risks with other acceptable risks in society, including in particular those arising from other (non-nuclear) pollutants.

I should like to make some comments in each of these three areas.

First, on the question of dose-effect relations. These are our principal means of extrapolation to low doses, and we have to rely mainly on empirically developed relationships, such as those of Sacher and Grahn with respect to the mouse (now being applied with, fortunately, rather similar results in important experiments by Norris with respect to the dog). These relations show some interesting changes in slope at low doses but much more data are needed for a thorough documentation. This type of empirical relation is supplemented by theories such as that of dual radiation action by Kellerer and Rossi. Theories of this type serve two distinct functions: (a) they provide us with a framework into which proposed mechanisms of biological damage must fit, i.e. speculation about the nature of the damage process must be consistent with theory. (Here one may comment parenthetically that carcinogenesis, and not cell-killing, is our principal endpoint at low doses, and it seems unlikely that a single, initial molecular event is responsible for both. Separating these two phenomena, and the factors associated with them, is an important matter for clarification in some cases.) (b) These theories enable us to make predictions for low doses — and Rossi and Kellerer's theory certainly provides us with some estimates of RBE for different radiations at low doses that cannot be ignored (as Dobson has emphasized by stating that we should increase the QF for tritium β particles). However, this question is intimately tied up with the effects of dose rate. High RBEs for high LET radiations at low doses may

only be an expression of the fact that dose rate factors are minimal at high LET and substantial at low LET — substantial in this case meaning a factor of somewhere between one and ten.

Thus, in applying this to radiation protection, we have the choice of recognizing that there is no proportionality between high and low doses in the case of γ radiation and attempting to quantitate this fact, or of accepting a proportional relationship for γ rays and adjusting QFs upwards to account for the high RBEs predicted by Rossi and Kellerer and now observed in many biological systems at low doses. New information on these points has been presented at this symposium (Dobson's ^3H β are an example) but the protection bodies (ICRP, NCRP, etc.) will have to come to grips with the question in a serious fashion.

It should be noted here that the theory of dual action, in the form considered here, does not include a time factor — a factor, incidentally, which could easily vary over a substantial range with endpoint and with species; the introduction of an appropriate time factor might make it possible to account for dose-rate factors and relate these to radiation quality.

The second area, that of extrapolation from other biological systems to man, has received considerable attention here. Both for external radiation (Norris, dogs; Erickson, pigs; Hupp, goats; etc.) and for internal radiation (Rosenblatt, ^{226}Ra and ^{239}Pu ; Jee) attempts are being made to observe and quantitate phenomena in species intermediate between mouse and man, such as the dog. The mouse/dog/man relationship has been carefully considered for ^{226}Ra and ^{239}Pu , but much more needs to be done to establish these interspecies relations better, both for external radiation and for various internal emitters, with the differences in metabolism that may be involved.

Reference has also been made to epidemiological data in man, mostly for exposures at or near the natural background. Changes in natural background, in general, do not show changes in response identifiable with the radiation exposure, and according to Frigerio these should have been seen; in fact, he believes a negative correlation exists between dose and effect. Such studies are crude at present, but when adequately refined, they may set a lower limit to levels at which human effects occur.

The third area deals with the relationship of low-level effects to risks and to the setting of standards. The radiation field has traditionally ignored other fields in setting its standards. Regrettably, it has not come to grips with the basic question of how to set standards in relation to natural background. A committee of the National Council for Radiation Protection under the Chairmanship of H. Friedell as long ago as 1959 suggested that standards should be based on, or at least related to, the natural background. This suggestion has still not been implemented.

If we do base our standards on the natural background, what sort of basis do we choose? If we can live, as we do, with the natural background, can we live with twice the natural background? Is this idea tenable? Or should we be more, or less, restrictive?

Should we follow Weinberg and Adler (Congressional Testimony for Subcommittee on Environment and the Atmosphere (Chairman: Brown), House Science and Technology Committee, 7 November 1975), who recently suggested that we restrict additional human exposure to no more than the standard deviation of the background (i.e., ~ 20 mrem/a), on the grounds that we could never hope to see effects at this level even if there are any? One can hardly imagine being more restrictive than that. If we

accept, say $1 \times N$ (or $0.2 N$) for the population, then what about occupational exposures? Should they be higher by an acceptable level (e.g. $\times 10$, rationale)? Should they be based directly on occupational experience, and is there enough information for this to be done? Once we approach the problem from the natural background level, occupational exposure levels take on a new perspective.

We seem to feel intuitively that we have time to deal with these questions and that our present standards are not too bad. This may be true, but frankly speaking, we are not moving rapidly towards a solution to these problems.

Furthermore, much hangs on what we do — other pollutants will demand more and more of the "total acceptable risk" to populations that we are willing to accept from pollutants. There are more and more of them to make a claim, and standard-setters dealing with them will look to the radiation field, formerly a good pilot in the experimental field at least, to guide them. At present, I think that we in the radiation field find ourselves in some disarray, or at least are not yet ready to answer some of the questions I have raised. But we must start tackling them. Since radiation will be our guide, we must look to the future for better experiments, tested models and epidemiological information — all, it is to be hoped, brought together and discussed at symposia such as this, so as to provide the base we need for future standards.

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The following conversion table is provided for the convenience of readers and to encourage the use of SI units.

FACTORS FOR CONVERTING UNITS TO SI SYSTEM EQUIVALENTS *

SI base units are the metre (m), kilogram (kg), second (s), ampere (A), kelvin (K), candela (cd) and mole (mol).

[For further information, see International Standards ISO 1000 (1973), and ISO 31/0 (1974) and its several parts]

<i>Multiply</i>	<i>by</i>	<i>to obtain</i>
Mass		
pound mass (avoirdupois)	1 lbm = 4.536×10^{-1}	kg
ounce mass (avoirdupois)	1 ozm = 2.835×10^1	g
ton (long) (= 2240 lbm)	1 ton = 1.016×10^3	kg
ton (short) (= 2000 lbm)	1 short ton = 9.072×10^2	kg
tonne (= metric ton)	1 t = 1.00×10^3	kg
Length		
statute mile	1 mile = 1.609×10^0	km
yard	1 yd = 9.144×10^{-1}	m
foot	1 ft = 3.048×10^{-1}	m
inch	1 in = 2.54×10^{-2}	m
mil (= 10^{-3} in)	1 mil = 2.54×10^{-2}	mm
Area		
hectare	1 ha = 1.00×10^4	m ²
(statute mile) ²	1 mile ² = 2.590×10^0	km ²
acre	1 acre = 4.047×10^3	m ²
yard ²	1 yd ² = 8.361×10^{-1}	m ²
foot ²	1 ft ² = 9.290×10^{-2}	m ²
inch ²	1 in ² = 6.452×10^{-2}	mm ²
Volume		
yard ³	1 yd ³ = 7.646×10^{-1}	m ³
foot ³	1 ft ³ = 2.832×10^{-2}	m ³
inch ³	1 in ³ = 1.639×10^{-4}	mm ³
gallon (Brit. or Imp.)	1 gal (Brit) = 4.546×10^{-3}	m ³
gallon (US liquid)	1 gal (US) = 3.785×10^{-3}	m ³
litre	1 l = 1.00×10^{-3}	m ³
Force		
dyne	1 dyn = 1.00×10^{-5}	N
kilogram force	1 kgf = 9.807×10^0	N
poundal	1 pdl = 1.383×10^{-1}	N
pound force (avoirdupois)	1 lbf = 4.448×10^0	N
ounce force (avoirdupois)	1 ozf = 2.780×10^{-1}	N
Power		
British thermal unit/second	1 Btu/s = 1.054×10^3	W
calorie/second	1 cal/s = 4.184×10^0	W
foot-pound force/second	1 ft·lbf/s = 1.356×10^0	W
horsepower (electric)	1 hp = 7.46×10^2	W
horsepower (metric) (= ps)	1 ps = 7.355×10^2	W
horsepower (550 ft·lbf/s)	1 hp = 7.457×10^2	W

* Factors are given exactly or to a maximum of 4 significant figures

<i>Multiply</i>	<i>by</i>	<i>to obtain</i>
Density		
pound mass/inch ³	1 lbm/in ³ = 2.768 × 10 ⁴	kg/m ³
pound mass/foot ³	1 lbm/ft ³ = 1.602 × 10 ¹	kg/m ³
Energy		
British thermal unit	1 Btu = 1.054 × 10 ³	J
calorie	1 cal = 4.184 × 10 ⁰	J
electron-volt	1 eV ≈ 1.602 × 10 ⁻¹⁹	J
erg	1 erg = 1.00 × 10 ⁻⁷	J
foot-pound force	1 ft·lbf = 1.356 × 10 ⁰	J
kilowatt-hour	1 kW·h = 3.60 × 10 ⁶	J
Pressure		
newtons/metre ²	1 N/m ² = 1.00	Pa
atmosphere ^a	1 atm = 1.013 × 10 ⁵	Pa
bar	1 bar = 1.00 × 10 ⁵	Pa
centimetres of mercury (0°C)	1 cmHg = 1.333 × 10 ³	Pa
dyne/centimetre ²	1 dyn/cm ² = 1.00 × 10 ⁻¹	Pa
feet of water (4°C)	1 ftH ₂ O = 2.989 × 10 ³	Pa
inches of mercury (0°C)	1 inHg = 3.386 × 10 ³	Pa
inches of water (4°C)	1 inH ₂ O = 2.491 × 10 ²	Pa
kilogram force/centimetre ²	1 kgf/cm ² = 9.807 × 10 ⁴	Pa
pound force/foot ²	1 lbf/ft ² = 4.788 × 10 ¹	Pa
pound force/inch ² (= psi) ^b	1 lbf/in ² = 6.895 × 10 ³	Pa
torr (0°C) (= mmHg)	1 torr = 1.333 × 10 ²	Pa
Velocity, acceleration		
inch/second	1 in/s = 2.54 × 10 ¹	mm/s
foot/second (= fps)	1 ft/s = 3.048 × 10 ⁻¹	m/s
foot/minute	1 ft/min = 5.08 × 10 ⁻³	m/s
mile/hour (= mph)	1 mile/h = $\begin{cases} 4.470 \times 10^{-1} \\ 1.609 \times 10^0 \end{cases}$	$\begin{cases} \text{m/s} \\ \text{km/h} \end{cases}$
knot	1 knot = 1.852 × 10 ⁰	km/h
free fall, standard (= g)	= 9.807 × 10 ⁰	m/s ²
foot/second ²	1 ft/s ² = 3.048 × 10 ⁻¹	m/s ²
Temperature, thermal conductivity, energy/area·time		
Fahrenheit, degrees - 32	$\left. \begin{matrix} ^\circ\text{F} - 32 \\ ^\circ\text{R} \end{matrix} \right\} \begin{matrix} 5 \\ 9 \end{matrix}$	$\left\{ \begin{matrix} ^\circ\text{C} \\ \text{K} \end{matrix} \right.$
Rankine		
1 Btu·in/ft ² ·s·°F	= 5.189 × 10 ²	W/m·K
1 Btu/ft·s·°F	= 6.226 × 10 ¹	W/m·K
1 cal/cm·s·°C	= 4.184 × 10 ²	W/m·K
1 Btu/ft ² ·s	= 1.135 × 10 ⁴	W/m ²
1 cal/cm ² ·min	= 6.973 × 10 ²	W/m ²
Miscellaneous		
foot ³ /second	1 ft ³ /s = 2.832 × 10 ⁻²	m ³ /s
foot ³ /minute	1 ft ³ /min = 4.719 × 10 ⁻⁴	m ³ /s
rad	= 1.00 × 10 ⁻²	J/kg
roentgen	R = 2.580 × 10 ⁻⁴	C/kg
curie	Ci = 3.70 × 10 ¹⁰	disintegration/s

^a atm abs: atmospheres absolute;
atm (g): atmospheres gauge.

^b lbf/in² (g) (= psig): gauge pressure;
lbf/in² abs (= psia): absolute pressure.

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